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Editor

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See McBride, *J. D. Res.* **16**: 537, 1937.

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EDITOR'S VIEWPOINT

Grossman and Dannenberg (p. 435) give the results of an interesting industrial hygiene study. They measured the concentration of mercury vapor in 50 dental offices and found it far below the level of toxicity. Greater concentrations were found in offices where the dentists used greater quantities of mercury. The problem of mercury toxicity in relationship to dentistry is introduced from time to time. Most studies have been concerned with the possibility of mercurial poisoning from amalgam fillings rather than poisoning from the more toxic mercury vapor. Although it appears that poisoning from the vapor in dental offices or from amalgam fillings is extremely remote, it should be remembered that mercury is one of the many toxic substances with which the dentist is concerned.

Healey and Phillips (p. 439) present an interesting study of 1,521 defective amalgam restorations. Of these, 56 per cent were due to improper cavity preparation and 40 per cent to improper handling of amalgam, both factors under the control of the operator. Standardized alloys, resulting from efforts of the Research Commission and the Bureau of Standards, have removed the old hazard of material failure and the myth of soft teeth was exposed years ago. Perhaps if all dentists required themselves to report five- and ten-year "cures" on each restoration, in a manner similar to cancer treatment evaluation, there might be fewer controllable failures.

A series of studies of eight different brands of hydrocolloid impression materials by James (p. 447) showed expansion and contraction of the impressions resulting in oversized and undersized dies. It is suggested that fillers be investigated more thoroughly as all of the materials have a common base. Most research in this field has been left to the manufacturers. As in all research, independent studies of the same subject in different laboratories, commercial, institutional, and individual, should be encouraged.

Dwight (p. 456) describes a method devised for electroplating artificial stone with copper. The technic depends on the displacement of iron by copper and should offer many uses other than plating of inlay dies.

Pindborg (p. 460), observing oral conditions and tobacco consumption in 5,690 Danish Royal Marines, has made a statistical correlation which indicates that tobacco has an adverse effect on the gingivae. The finding of a correlation between tobacco use and the occurrence of ulceromembranous (necrotizing ulcerative) gingivitis lends support to the empirical recommendation to abstain from smoking during treatment of the disease. In a further study of the same group of Danish Marines, Frandsen and Pindborg (p. 464) found a greater deposition of calculus in pipe smokers than in cigarette smokers. They suggest that this may result from greater salivation of pipe smokers.

Barr (p. 466) analyzed the clinical and radiographic data on caries occurrence on 20,000 proximal tooth surfaces. He observed, as had others, a bilateral symmetry of caries in the group picture but points out that in any one individual case the assumption of bilateral symmetry cannot be applied rigidly. In one-third of 117 individuals with intact dentitions, posterior proximal caries occurred in the absence of any maxillary anterior caries; while in the remaining two-thirds, the regional caries attack was correlated. Approximation appeared to be an important factor in caries attack of mesial and distal surfaces. The problem of missing teeth, which appears to plague all students of caries attack rate, is discussed and analyzed. Patterns of tooth surface susceptibility were developed. An interesting observation, which confirms the conventional practice and teaching in this country, was that "spot" fillings became defective five times more frequently than two-surface restorations. A possible selective factor here may have been the skill and carefulness of the operators following the conventional teachings.

Gruber (p. 483) investigated the oral conditions of 200 individuals with tuberculosis. He found no evidence for the opinion that there is a characteristic "tuberculosis type" of dental caries, nor for any belief that dental caries, gingivitis, or periodontal disease are more common in the patient with tuberculosis than in the person free of tuberculosis. The study illustrates the need for replacement of opinion by fact, and also shows a relatively simple but useful type of clinical investigation that can be carried out by dentists in institutions or private offices.

Reduction of experimental conditions to a single factor or a single group of factors is often more difficult than it may appear. Shaw (p. 495), noting an apparent difference in caries activity in littermate cotton rats, observed accumulations of wood fibers and debris in the sulci of the teeth of the cotton rats with less decay. Experimental study, reported here, shows that cotton rats raised in cages with sheet metal floors covered with wood shavings have fewer cavities from those raised in cages with screen flooring. Shaw does not explain the mechanism which results in this significant difference.

Crowell and Turner (p. 499), having demonstrated the production of oxalic acid in vitro from starch through the action of saliva of twelve individuals with clinically active caries, placed teeth in oxalic acid. Calcium ions were removed from the teeth in vitro, and calcium oxalate was deposited. The authors suggest that for individuals with certain types of amylase system, starch may be an important source of oxalic acid. They believe that the tooth and food sources could be robbed of their calcium ions, and calcium oxalates deposited in the arteries, ureter, or elsewhere. While the theory appears plausible, the clinical demonstration of the process that they have shown to take place, in vitro, in three weeks must be awaited.

Observing that old cultures of saliva in phenol red glucose broth returned to alkalinity, Snyder and Clarke (p. 503) sought out the cause. When the glucose became exhausted, yeasts with proteolytic ability released alkaline products which neutralized and then alkalized the media. The authors hint at a possible action of this type as an in-vivo neutralizer of acid activity or in the so-called proteolytic theory of caries. No claims are made but it is mentioned that the possibilities are being studied.

Hess and Smith (p. 507) determined the ascorbic acid content of the saliva from thirty-one caries-free individuals, and from fifty-four individuals with caries. They found no significant difference in the salivary ascorbic acid content of these two groups. Unfortunately, although the method for chemical analysis was carefully selected, the authors did not determine the caries activity of the individuals used. The mere absence of presence of cavities is insufficient evidence of the status of the process of caries. There are two general methods for evaluating the actual activity: (1) repeated thorough oral examinations with actual observation of progress or stasis of carious lesions, and (2) caries susceptibility tests such as the salivary lactobacillus index. It is unfortunate that Drs. Hess and Smith's dental advisors did not point this out. The authors have shown a lack of relationship between salivary ascorbic acid and freedom from, or presence, of cavities—not necessarily a lack of correlation between caries activity and salivary ascorbic acid, although the latter may be inferred.

Losee and Hess (p. 512) analyzing the protein content of various portions of enamel and enamel cuticle found from 0.05 per cent (cuticle) to 0.45 per cent (total stained enamel) protein. They found cystine, methionine, and phenylalanine in all portions of enamel and cuticle. Although enamel cuticle had the highest cystine content, it was far below the quantity found in true keratins. Further analyses for other amino acids are necessary to determine whether the proteins of enamel, which have been loosely called keratins, are true or pseudokeratins. The report of Block, Horwitt, and Bolling should be considered.

Too often those making analyses of enamel protein fail to differentiate surface accumulations of proteins from the basic organic matter of enamel, lumping all material received from the surface and from within the enamel. Block, Horwitt, and Bolling (p. 518) compared "brown pellicle," enamel proteins, and herring scales. They classify enamel proteins as eukeration, brown pellicle as a type of pseudokeratin or denatured mucoprotein, and herring scales as gelatin (similar to human dentin) and ichthylepidin.

Waerhaug (p. 525) submits a method for rapid decalcification of bone and tooth material. The technic utilizes the vacuum principle, removing carbon dioxide and permitting more intimate contact with the acid. The author found that decalcification time was reduced to approximately one-tenth the time used in conventional methods. The value of the time reduction is evident to the pathologist as well as the histologist.

H. B. G. R.

AMOUNT OF MERCURY VAPOR IN AIR OF DENTAL OFFICES AND LABORATORIES

LOUIS I. GROSSMAN, D.D.S., D.M.D., AND JAMES R. DANNENBERG, D.D.S.

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MERCURY is used almost daily by all dentists, with few exceptions. From time to time dentists have been concerned with the effects of their own well-being of repeated contact with this element. Even those whose operative work is mainly of the gold inlay or porcelain variety may use mercury in preparing amalgam dies and are constantly exposed to its vapors when grinding or polishing. Since mercury is such an ever-present factor in the operating room or laboratory of the dentist, it seemed desirable to determine if enough mercury vapor is present in dental offices to be harmful.

HISTORICAL

In 1810 an English ship left Spain laden with about 100 tons of mercury contained in leather pouches. On the way to its destination, the seams of the pouches ripped, spilling the mercury in the hold of the vessel. Vaporization of the mercury took place and the 200 passengers and crew were poisoned by the mercury vapor, resulting in two deaths and marked gingivostomatitis in all the others. All animals on board died of the mercury vapor before the ship reached England.

According to Giese,[1] some well-known scientists, among them Faraday and Pascal, were victims of chronic mercury poisoning even though they were not aware of it at the time. The more common symptoms of chronic poisoning are headache, irritability, respiratory irritation, metallic taste, and soft swollen gums.

Apparently the possibility of mercury vapor, from various sources, being toxic to humans has interested the medical and dental professions for a long time. In 1931 Souder and Sweeney[2] stated: "Discussions of the possibility of mercury poisoning from dental amalgams have been appearing in the medical, dental, and popular literature for several years. As a part of the dental research program at the National Bureau of Standards, it was decided to make quantitative determinations of the amounts of mercury available, under normal conditions, from the types of amalgam used in dental restorations." Their work was precipitated by the persistent claims of Stock[3] that mercury was being set free from amalgam fillings in minimal amounts but sufficient to cause chronic mercurial poisoning. He based his claim on the fact that mercury, in appreciable quantities, had been isolated by him from the urine of subjects who had one or more amalgam fillings in their mouths. Stock had previously found that a concentration of mercury of from 0.001 to 0.01 mg. per cubic meter of air was enough to cause mercury poisoning.

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Subsequent studies have shown that even those without amalgam fillings in their mouths may have traces of mercury in the urine because of the ingestion of certain foodstuffs containing mercury. To date, however, no study has been made of the amount of mercury vapor in the air of dental offices to determine whether it is a hazard to health.

PROCEDURE

The assaying of the amount of mercury vapor in air was made in the operating rooms and laboratories of dentists and in several commercial laboratories. Mercury, in the form of its vapor present in the air, was measured. The vapor was chosen as the medium because: (1) ease of measurement; (2) it is the form most easily absorbed by the body and is therefore most harmful; (3) the toxic limits of mercury vapor have been established.

The apparatus used in this study was a portable General Electric mercury-vapor detector of the instantaneous type. It operates on the principle of light absorption, i.e., ultraviolet rays are scattered when they pass through an atmosphere containing mercury vapor.[4]

Essentially the apparatus used consisted of a cylindrical chamber housing an ultraviolet lamp at one end and a phototube at the other. A small blower within the apparatus forced air from the atmosphere through the chamber at a constant rate per minute. The phototube controls the voltage which is balanced manually. When mercury vapor is introduced into the chamber, the amount of ultraviolet light is reduced causing a measurable change in the voltage, as indicated on the instrument panel. Since the apparatus was carried about and therefore subject to slight alteration of the standardizing dials, the readings could be considered accurate to only one scale division.

The apparatus is sensitive enough to measure mercury-vapor concentrations from 0.03 to 3.0 milligrams per cubic meter of air, or from 0.004 to 0.37 parts per million by volume with an accuracy of approximately 5 per cent. It has been established[4] that the toxic limit is 1.3 parts of mercury vapor in 100,000,000 parts of air by volume, or 0.1 mg. of vapor in one cubic meter of air.

The mercury-vapor detector was operated simply by plugging it into the ordinary electric outlet and calibrating the apparatus. The reading shown on the instrument panel was interpolated from a calibration curve in parts per million of mercury vapor. The volume of mercury vapor detected in dental offices and laboratories was found to be so low in the scale, however, that interpolation was not practicable. Instead, the meter readings were compared with the meter reading of a minimum toxic amount of mercury vapor, namely 50 (Fig. 1). For simplicity, therefore, results will be expressed in terms of actual meter readings.

The average findings for dental operating rooms was 3.2, with a high of 12 and a low of 0. Dental laboratories associated with dental offices averaged 4.1, with a high of 6 and a low of 2. Commercial laboratories averaged 8.6. As was to be expected, orthodontists' offices showed lower readings (average

2.0) than those of general practitioners. On the other hand, dentists who acknowledged using a considerable amount of mercury had offices whose air gave considerably higher readings. It was observed that concentrations of mercury vapor varies in the same offices depending upon the position of the apparatus. Readings of specimens obtained close to the floor were slightly higher than those at eye level, showing that the greater concentration of mercury vapor is near the floor where the mercury is likely to fall and be vaporized. Unfortunately, the study was carried out during the springtime when radiators and heaters, which might have increased the volatilization of mercury, were not in use.

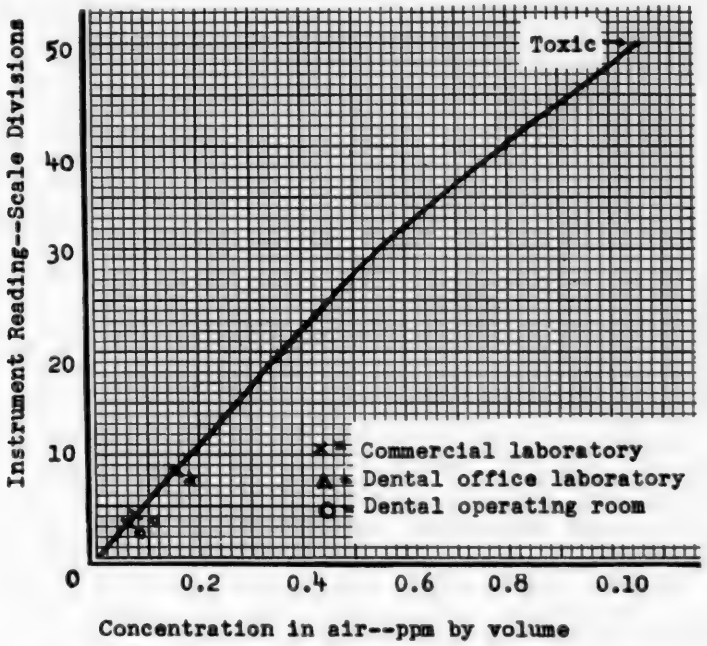

Fig. 1.—Concentration of mercury vapor in dental offices and laboratories. Toxic level of mercury vapor is indicated at top, right. Average amounts for commercial laboratories is indicated by cross, for dental office laboratories is indicated by triangle, and for dental operating rooms is indicated by circle in lower left hand corner.

CONCLUSION

The amount of mercury vapor with which the average dentist comes into contact in his office or laboratory is not toxic. Concentrations of the vapor were so uniformly low that the study was terminated after fifty dental offices were examined instead of the one hundred originally planned. It is interesting to note, however, that slight (but nontoxic) amounts of mercury vapor are present in the air of dental offices, and that the concentration of mercury vapor is directly proportional to the amount of mercury used by the individual dentist.

The authors are indebted to the General Electric Co. for the loan of the apparatus used in this study, and to Dr. Gordon R. Winter and Dr. James B. Davidson, Jr., for many helpful suggestions.

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A CLINICAL STUDY OF AMALGAM FAILURES

HARRY J. HEALEY, A.B., D.D.S., AND RALPH W. PHILLIPS, B.S.

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ROUTINE mouth examinations impress upon the dentist the frequency with which dental amalgam is used as a material for restoring tooth structure lost as a result of caries. There is no doubt that with conscientious use, amalgam is an excellent filling material. Brekhus and Armstrong observed that not only was amalgam the most frequently used material in restorative dentistry, but also there was a lower percentage of failures in restorations made with amalgam than with any of the other restorative materials.[1] However, daily observations in the dental office do reveal many amalgam failures.

On the basis of the specifications established by the Research Commission of the Bureau of Standards, manufacturers are now selling the profession very few definitely inferior dental alloys; hence, the observed amalgam failures must be attributed to factors other than the material itself. The success of the amalgam restoration is dependent upon control of, and attention to, many variables. Everything that is done from the time that the cavity is prepared until the restoration has been polished has a definite effect upon the physical properties and the success or failure of the restoration. The objective of this investigation was, therefore, to analyze the causes of failure in a large number of amalgam restorations. No attempt was made to determine the percentage of failure in relation to the total number of restorations examined.

Several reports of studies of amalgam failures have been made previously,[2, 3, 4] but none have systematically itemized the various causes of failure; therefore, it seemed wise to make a careful clinical evaluation and comparison of the different variables involved in the use of amalgam as a restorative material. Such data could be valuable in judging the relative importance of all of the factors which help to make up an exacting amalgam technic.

SOURCE OF DATA

Data were accumulated by the tabulation of causes of failure detected by visual examination of 1521 defective amalgam restorations in the mouths of patients who presented themselves for treatment in the Operative Dentistry Clinic, Indiana University School of Dentistry. The history of these restorations was unknown; thus, no information was available concerning the manipulative procedures and technics employed or the conditions under which they were inserted. A restoration was judged to be defective if it needed replacement due to its failure to restore, or if it seriously interfered with the normal function and integrity of the tooth. The main criterion for this evaluation was the existence of recurrent caries or those conditions which are known to be

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the forerunner of recurrent caries. Some of the defective restorations had more than one cause of failure; in these cases only the predominant cause was tabulated. All mouth examinations were made by the same operator (H.J.H.), using a mouth mirror, sharp explorer, and air syringe. It should be pointed out that this was an objective study and, as any clinical investigation, must involve some human error.

Clinical observations preliminary to the actual accumulation of the data revealed that failure of amalgam restorations was generally due to: (1) fracture, (2) dimensional change, (3) recurrent caries, and (4) pulp or periodontal involvement. Various factors contributed to these observed types of failures. A data card was compiled on which all of these factors were enumerated. Upon examination, the type of failure for each defective amalgam was checked and the causative factor indicated; for example, a *fractured* amalgam which occurred because of *improper manipulation.*

FINDINGS

Recurrent caries.—The composite data showing the four general types of failures and the causative factors for each type can be seen in Table I. The most common cause of failure was recurrent caries. Actual existence of recurrent caries was found in 813 fillings or 53.5 per cent of the total number of failures observed. These data are summarized in graphic form in Fig. 1, which shows that considerable more amalgams failed because of recurrent caries than because of fracture, dimensional change, or pulp or periodontal involvement.

TABLE I

TYPES OF FAILURES OBSERVED AND THE CAUSATIVE FACTORS CONTRIBUTING TO THEM

CAUSE OF FAILURE	NUMBER OF FILLINGS
Fracture	
Faulty manipulation	325
Cavity preparation	66
Traumatic occlusion	7
	398
Dimensional change	
Expansion	253
Contraction	38
	291
Recurrent caries	
Cavity preparation	786
Failure of material	27
	813
Pulp or periodontal involvement	
No cement base	3
No restoration of normal anatomic contour	16
	19

Factors contributing to failures due to recurrent caries were adjudged to be: first, improper cavity preparation, and second, failure of the restorative material itself. Recurrent caries due to failure of the material means restorations by amalgam when amalgam was not indicated; for example, recurrent caries around a proximal incisal amalgam, particularly on the lower anterior teeth.

Improper cavity preparation was found to be the causative factor in 786 restorations, while use of amalgam where contraindicated accounted for only 27 failures. It is recognized that some cases of recurrent caries might have been due to caries not completely removed at the time of the amalgam cavity preparation. Even if it were possible to ascertain such cases, they, nevertheless, would have to be listed under improper cavity preparation.

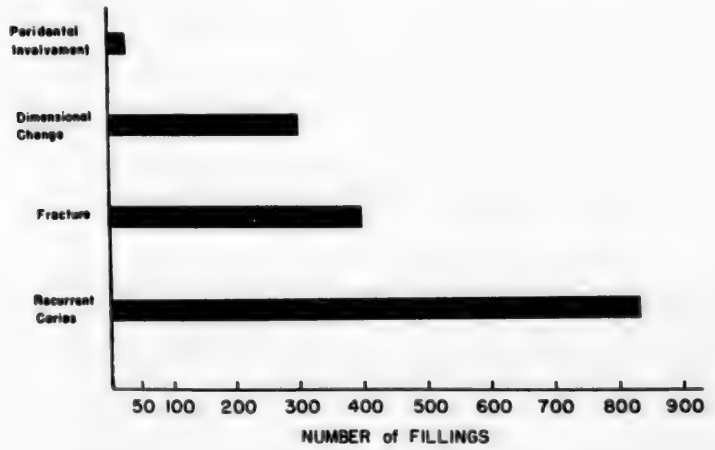

Fig. 1.—Numerical comparison of observed types of failures.

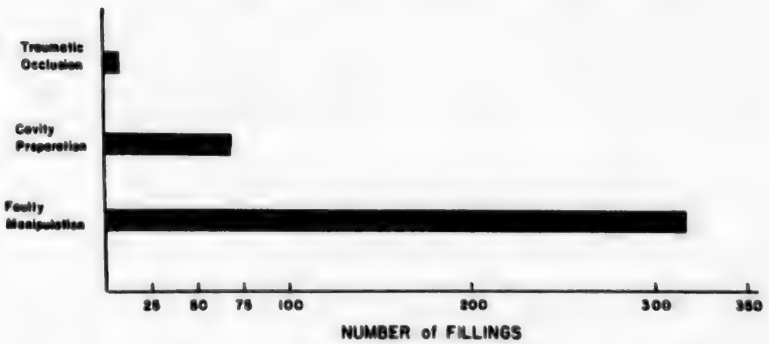

Fig. 2.—Comparison of causative factors contributing to fracture.

Fracture.—Fracture was observed in 398 of the defective restorations or in 26.2 per cent of all failures examined. The greatest contributing factor in these (Fig. 2) was faulty manipulation of the material. Three hundred twenty-five of the 398 which failed due to fracture were attributed to faulty manipulation with cavity preparation and occlusal trauma causing failure in a lesser number of restorations. It was felt that the cavity preparation in these failures due to faulty manipulation was adequate and that if a properly manipulated amalgam had been placed in the preparation, no fracture would have occurred.

This had to be, of course, a purely objective evaluation dependent upon the judgment of the examiner.

Various manipulative factors at the time of insertion could have lowered the strength of the amalgam sufficiently to produce subsequent fracture. It was impossible to determine by visual observation just what variable in the manipulative procedure had been involved in these fractured restorations, but those variables which could have caused loss in strength and resulting fracture are: (1) improper mercury-alloy ratio, (2) undertrituration, and (3) improper condensation. Previous study has clearly indicated that all of these factors will alter markedly the crushing strength.[5, 6, 7] Typical of this kind of fracture, due apparently to faulty manipulation, is that seen in Fig. 3. The restoration was placed in a cavity preparation which apparently provided sufficient bulk of amalgam for strength. However, fracture did occur, undoubtedly induced by one, two, or all of the manipulative variables listed above.

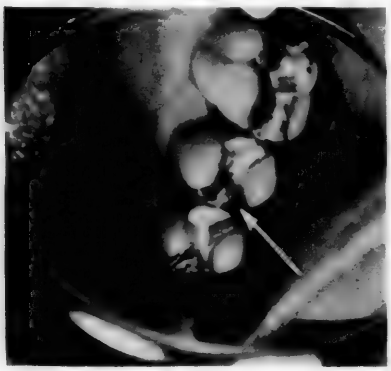

Fig. 3.

Fig. 4.

Fig. 3.—Fracture due to faulty manipulation.
Fig. 4.—Fracture due to insufficient cavity depth.

From Table I, it can be noted that only 66 restorations which fractured did so because of improper cavity preparation. These fractures included those which exhibited serious marginal breakdown in the proximal flare and occlusal areas. The causative factor in these cases was attributed to improper preparation of the cavosurface area resulting in inadequate provision for bulk of material. Another type of failure observed was that due to insufficient cavity depth, an example of which is seen in Fig. 4.

Evidence of occlusal trauma causing fracture was observed on only a very few restorations and in these failures, evidence of such traumatic occlusion in the form of shiney facets was observed on the remaining or intact portions of the restorations.

Flow was observed in some cases but not of sufficient magnitude to interfere with the effectiveness of the restoration. It is true that some of the fail-

ures due to fracture might have been hastened by flow, particularly on restorations with narrow isthmuses. Flow in these areas would further narrow the bulk and make it more susceptible to fracture.

Dimensional change.—Substantiation of previous investigations[7, 9, 10] relative to excessive expansion of amalgam was obtained in this study. It was observed that this expansion accounted for the failure in 16.6 per cent of all the defective restorations examined and those conditions assumed to be contraction in only 2.5 per cent of the total.

Two examples of excessive expansion are shown in Figs. 5 and 6. The severe expansion exhibited in these restorations must be attributed to delayed expansion due to moisture contamination. Such contamination might occur from mulling in a perspiring palm, packing into a wet cavity, or saliva incorporated during condensation. This type of failure emphasizes the care that must be exercised in the handling of amalgam to prevent this tremendous delayed expansion. Such expansion is usually the forerunner of recurrent caries.

Fig. 5.

Fig. 6.

Figs. 5 and 6.—Expansion probably caused by moisture contamination.

The failures due to contraction of the restoration, which were definitely in the minority, can be attributed either to overtrituration of the alloy or to possible insufficient condensation. In extremely narrow fissures, feather edges are often observed. Later these extensions break off and carry away some of the amalgam. The result is a so-called "ditched" filling which may appear to be due to contraction of the alloy. Since previous studies have indicated that contraction due to overtrituration is not too readily observed clinically,[11, 12] the restorations observed here are possibly due to improper condensation in small fissues.

Pulp or periodontal involvement.—In only 1.2 per cent of all the defective restorations observed was replacement necessary because of morbid effects on the pulp and/or the periodontal tissues. Contributory factors in this kind of failure were pulpal irritation due to the absence of protective insulation of the deep portion of the cavities, abnormal proximal contours, and cervical excesses. Fig. 7 shows a radiograph of cervical excess which resulted in periodontol involvement.

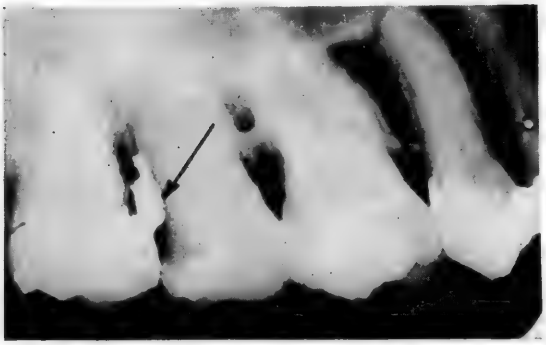

Fig. 7.—Radiograph showing periodontal involvement due to cervical excess of amalgam.

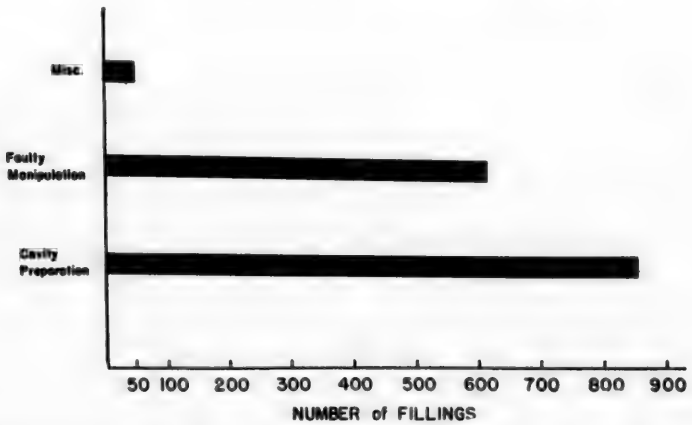

Fig. 8.—Improper cavity preparation and faulty manipulation as principal causes of failure.

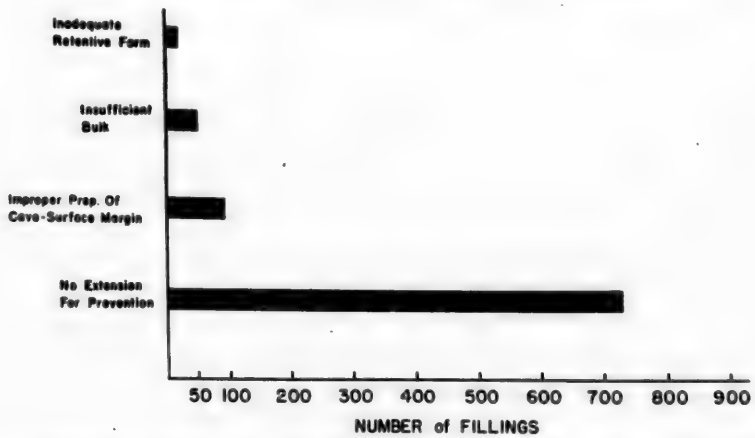
Inadequate Retentive Form
Insufficient Bulk
Improper Prep. Of Cavo-Surface Margin
No Extension For Prevention
50 100 200 300 400 500 600 700 800 900
NUMBER of FILLINGS

Fig. 9.—Insufficient extension for prevention, the most common error in amalgam cavity preparation.

CAVITY PREPARATION AS A FACTOR IN FAILURE

It has been shown in this report that improper cavity preparation, contributing to (1) actual recurrence of caries and (2) fracture, was the greatest single factor in the failures which we examined (Fig. 8).

These data verify the opinions of Crawford[2] and Easton[4] in regard to the importance of proper cavity preparation in the ultimate clinical success of the amalgam restoration. However, from this summary it can be seen that proper manipulation does play a vital role, inasmuch as 40.5 per cent of all the restorations examined failed because of this factor.

TABLE II

COMPARISON OF THE FREQUENCY OF VIOLATIONS OF THE FUNDAMENTAL PRINCIPLES OF AMALGAM CAVITY PREPARATION

CAUSES OF FAILURE	NUMBER OF FILLINGS
No extension for prevention	
a. Proximal flares	244
b. Occlusal fissure	377
c. Class V extension	97
	718
Insufficient provision for bulk	
a. At isthmus	21
b. Occlusal fissure	5
c. Cavity depth	19
d. Cusp cavity	2
	47
Improper preparation of cavosurface margin	80
Inadequate retentive form	7

Fig. 10.—Amalgam failure due to improper extension of occlusal fissures.

Frequency with which violation of the fundamental principles of amalgam cavity preparation occurred was found to be, as seen in Table II: (1) failure to extend cavity margins to relatively immune areas, (2) insufficient provision for bulk of material, (3) improper preparation of the cavosurface margin, and (4) inadequate retentive form. These composite data are shown in graphic form in Fig. 9. These observations seem to bear out the statement of Roper: "If all the causes for all the faulty restorations with amalgam ever made were considered carefully, there would be no question that the most common would be this: insufficient removal of tooth structure."[13] An example of failure due to improper extension of occlusal fissures is shown in Fig. 10.

CONCLUSIONS

A group of 1521 defective amalgam restorations was studied for the purpose of determining their causes of failure.

It was found that the failures could be grouped into those due to: (1) actual recurrent caries, (2) fracture, (3) dimensional change, and (4) pulp and/or periodontal involvement; the frequency occurring in that order.

The main factors which contributed to the failures were judged to be (1) improper cavity preparation, which was the causative factor in 56 per cent of all the failures and (2) faulty manipulation of the amalgam or its contamination at the time of insertion, 40 per cent of all failures.

It is apparent from this study that the clinical success of amalgam restorations is dependent upon many factors, most of which can be controlled by the dentist. Careful cavity preparation, proper manipulation, and restoration of normal anatomical contour can reduce to a minimum clinical failure of amalgam.

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MAINTENANCE OF EQUILIBRIUM IN REVERSIBLE HYDROCOLLOID IMPRESSIONS

III. A Report on Tests Made With Five Stock Impression Materials

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INTRODUCTION

A PREVIOUS report[1] described the procedure used in testing impression materials. Dental gold castings which accurately fitted porcelain tooth preparations were made. The fit of these test castings to dies produced from impressions made with the various materials was the criterion of accuracy. In the first report[1] two stock materials were used, one of which contracted at a critical end-point time interval between making the impression and pouring the die, producing oversized dies thereafter; and another which expanded, producing undersized dies at the critical point. A second report[2] described the results obtained with materials supplied by manufacturers for testing, using the same procedures and same test castings. This paper describes the results obtained with other well-known commercial reversible hydrocolloid impression materials obtained in the open market, again using the same procedures and the same test castings.

MATERIALS AND METHODS

Table I gives the materials tested, identified with their code letters, together with the manufacturer's name.

TABLE I

MATERIALS*

CODE	BRAND NAME	MANUFACTURER
D	Surgident	Surgident, Inc. West Los Angeles, Calif.
E	Kerr Hydro-Colloid	Kerr Manufacturing Co. Detroit, Mich.
F	S. S. White Elastic Colloid	The S. S. White Dental Mfg. Co. Philadelphia, Pa.
G	Dentocoll	The L. D. Caulk Company Milford, Delaware
H	Coeloid 66	Coe Laboratories, Inc. Chicago, Ill.

*Deelastic, manufactured by Dee Division of Handy & Harman, Chicago, Ill., and Plasticoll, manufactured by Lee S. Smith & Son, Pittsburgh, Pa., were tested in the experiments reported in Part I.

D-P Impression Colloid, manufactured by Dental Perfection Company, Glendale, Calif., was reported in Part II, under *Materials Supplied by the Manufacturer for Testing.* Since the Dental Perfection Company has not yet placed on the open market the special syringe material used in the latter report, and the marketed material available is not adapted to injection through small syringes, it was necessary to omit D-P Impression Colloid in this study.

All materials were prepared and used in the same manner detailed in Part I.*

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*James, A. G.: *J. D. Res.* **28**: 108, 1949.

Models were all poured in artificial stone* from the same batch. The poured impression was placed in tap water, or in the case of one material, in Control Solution, or 2 per cent potassium sulfate for a period of at least one hour, the period of stone set. Following separation of the die from the impression the die was allowed 24 hours for complete drying before any test of the casting fit on the die was made.

Impressions of the M-O-D-L porcelain tooth preparation were made as described with each material and poured immediately. The dies produced from these impressions then acted as controls.

Impressions of the porcelain tooth preparation were again made with each of the materials and immersed in tap water for 15 minutes prior to pouring the models. Additional impressions were made with Material D and stored for 15 minutes before pouring the model in (1) 2 per cent potassium sulfate solution and (2) Surgident Control Solution. The 15 minutes' storage of impressions (between making and pouring the models) was included as part of the establishing of controls because this range of time covers the usual time intervals occurring in my office.

In the following tests described, when a deviation from accuracy was found, new material was used to duplicate the procedure as a check. In some instances material was reheated and reused as a further check, but these latter observations, although they suggest an interesting field for research in relative osmotic balances, are not reported in this paper.

Since the manufacturers of the other materials did not recommend other than water for storing the material, only Material D was placed in either potassium sulfate solution or Control Solution as supplied.

MATERIAL D TESTS

The manufacturer recommends placing the impression in "Control Solution" as supplied, and states:

"The impression may be left there until pouring up in stone (by control is meant control for thermal changes, control against drying out, control against swelling, as when placed in water, control against dimensional changes while setting of the stone, control of time elements). Impressions from evening or Saturday appointments may be left until the next working day. Never allow the impression to dry either before pouring or while the model is setting. Model stone (Hydrocal) will set perfectly while completely immersed in the solution."

Three impressions of the preparation were made with this material and poured immediately in artificial stone, then placed in (1) tap water, (2) Control Solution, (3) 2 per cent potassium sulfate solution, respectively, for the period of stone set. All of the dies so produced were oversize.

Three impressions were then made and stored for 15 minutes in water, Control Solution, 2 per cent potassium sulfate, respectively, before pouring.

*S.S.W. Albastone. The same amount of water was used for each mix and the powder added to the desired pattylike consistency. The volume of water used was constant and, while the powder was not weighed, the author nevertheless believes that since the same investigator made all mixes and in the event of too dry a mix discarded it rather than attempting to use it, the water-powder ratio of the hydrocal mixes was within a very narrow standard range and constituted an entirely satisfactory procedure for any research problem based on clinical conditions.

The die from the impression stored in water was oversize. That from the impression stored in the Control Solution was slightly oversize, by the evidence of friction in seating the casting. That from the impression stored in 2 per cent potassium sulfate (K_2SO_4) was accurate.

Three impressions were again made and placed for one hour in water, Control Solution, and potassium sulfate solution, respectively. That stored in water gave an accurate die; the die from the impression stored in the Control Solution was oversize, indicating contraction of the impression material during the storage period; that from the impression stored in the 2 per cent potassium sulfate solution was accurate.

Since the manufacturer stated accuracy could be maintained overnight, or longer, impressions were again made and placed respectively in the water, Control Solution, and potassium sulfate, for 19 hours. The die from the impression stored in water appeared accurate; the die produced from the impression stored in the Control Solution was decidedly oversize; and that produced from the impression stored in potassium sulfate was slightly oversize, giving slight friction to the seating of the casting.

Because this material displayed no consistent pattern and was so contrary to the advertised qualities, a supply of different batch was purchased from a different supply house and the tests were duplicated. Three impressions made of the preparation and poured immediately and then placed in water, Control Solution, and 2 per cent potassium sulfate, respectively, gave dies (1) oversize in water, (2) accurate in Control Solution, (3) oversize in potassium sulfate.

Three impressions made and stored 15 minutes in water, Control Solution, and potassium sulfate solution, respectively, for 15 minutes before pouring all gave accurate dies. Impressions again made and stored one hour before pouring in water, Control Solution, and potassium sulfate, respectively, all gave accurate dies. Impressions made and stored 19 hours in water, Control Solution, potassium sulfate, all gave oversized dies. The variables found in this material from batch to batch and its unpredictable behavior, make it appear unacceptable for accurate procedures (Table II).

TABLE II
MATERIAL D

TIME INTERVAL BETWEEN MAKING IMPRESSION AND POURING	IMPRESSION PLACED IN:	DIE RESULTS IMPRESSIONS*: NO. 1	NO. 2
Immediate	Water	+	+
	Control Solution	+	0
	2% K_2SO_4	+	+
Fifteen minutes	Water	+	0
	Control Solution	+	0
	2% K_2SO_4	0	0
One hour	Water	0	0
	Control Solution	+	0
	2% K_2SO_4	0	0
Nineteen hours	Water	0	+
	Control Solution	+	+
	2% K_2SO_4	+	+

*Accurate die = 0; oversize die = +; undersize die = −

MATERIAL E TESTS

Since the manufacturer of this material recommends only water for storing of the impressions, no salt solutions were used. Impressions with this material were made of the porcelain preparation as follows:

1. Poured immediately

Stored in water before pouring:

2. 15 minutes
3. 30 minutes
4. 45 minutes
5. 1 hour
6. 2 hours
7. 3 hours
8. 4½ hours
9. 16 hours
10. 17 hours

The impressions poured immediately produced an undersized die, therefore two impressions were again made and poured immediately. Each die so produced was found to be undersize. The impression stored 15 minutes in water before pouring produced an accurate die. The impression stored 30 minutes in water before pouring produced an undersized die, therefore another impression with new material was made and again stored in water 30 minutes before pouring. Again an undersized die was produced. The dies produced from the impressions stored 45 minutes, 1 hour, 2 hours, 3 hours in water were accurate. The die from the impression stored in water 4½ hours before pouring was undersize. Two impressions were made and placed in water for 16 and 17 hours, respectively, before pouring the dies. The dies so produced were both accurate in so far as the fit of the test casting to the dies could be determined by the sense of touch and careful examination with operating loupes (Table III).

TABLE III

MATERIAL E

TIME INTERVAL BETWEEN MAKING IMPRESSION AND POURING	IMPRESSION PLACED IN:	DIE RESULTS IMPRESSIONS*:		
		NO. 1	NO. 2	NO. 3
Immediate	Water	–	–	–
15 minutes	Water	0	0	–
30 minutes	Water	–	–	0
45 minutes	Water	0		
1 hour	Water	0	0	
2 hours	Water	0		
3 hours	Water	0		
4½ hours	Water	–	0	0
16 hours	Water	0		
17 hours	Water	0		

*Accurate die = 0.
Undersize die = –.
Oversize die = +.

MATERIAL F TESTS

The same procedure was followed with this material, impressions being made:

1. Poured immediately

Stored in water before pouring:

2. 15 minutes
3. 30 minutes
4. 45 minutes
5. 1 hour
6. 2½ hours
7. 3 hours
8. 4 hours
9. 5 hours

This material was found to break rather easily upon separation from the porcelain preparation. Fibers observed in the material appeared not to clog the needle of the small syringe as had been anticipated. The material before boiling had a grainy, coarse texture not observed in other materials. The die from the impression poured immediately gave slight friction to the seating of the casting, indicating slight oversize, but the die produced from the impression stored in water for 15 minutes before pouring was distinctly oversize, preventing the casting from being seated. The dies produced from the impressions stored 30 minutes, 45 minutes, 1 hour, 2½ hours, 3 hours, and 4 hours, were accurate in so far as careful examination showed. A die produced from an impression stored 5 hours in water was again distinctly oversize (Table IV).

TABLE IV
MATERIAL F

TIME INTERVAL BETWEEN MAKING IMPRESSION AND POURING	IMPRESSION PLACED IN:	DIE RESULTS IMPRESSIONS*:	
		NO. 1	NO. 2
Immediate	Water	+	+
15 minutes	Water	+	+
30 minutes	Water	0	+
45 minutes	Water	0	0
1 hour	Water	0	+
2½ hours	Water	0	0
3 hours	Water	0	
4 hours	Water	0	
5 hours	Water	+	+

*Accurate die = 0.
Oversize die = +.
Undersize die = −.

MATERIAL G TESTS

This material was found to be not well adapted for injection technic as it contains fibers and is very tough, necessitating reaming out the small needle of the syringe two or three times in the attempt to inject the cavity. Also it was noted that a good weld was not produced between the injected material

and the tray material. Impressions were made following the same procedure:

1. Poured immediately

Stored in water before pouring:

2. 15 minutes
3. 30 minutes
4. 45 minutes
5. 1 hour
6. 2 hours
7. 3 hours
8. 4 hours
9. 5 hours

The dies poured immediately and after 15, 30, and 45 minutes' storage in water, were all accurate. Those dies produced from the impressions stored in water one hour and beyond, through 5 hours, all were undersize in increasing degree. For recheck upon the method, impressions were again made and poured immediately, and after storage 15 minutes in water. The dies produced from these impressions were both accurate (Table V).

TABLE V
MATERIAL G

TIME INTERVAL BETWEEN MAKING IMPRESSION AND POURING	IMPRESSION PLACED IN:	DIE RESULTS IMPRESSIONS*:	
		NO. 1	NO. 2
Immediate	Water	0	0
15 minutes	Water	0	0
30 minutes	Water	0	0
45 minutes	Water	0	No weld
1 hour	Water	–	0
2 hours	Water	–	–
3 hours	Water	–	–
4 hours	Water	–	
5 hours	Water	–	

*Accurate die = 0.
Oversize die = +.
Undersize die = –.

MATERIAL H TESTS

Impressions were made with this material in the same manner:

1. Poured immediately

Stored in water before pouring:

2. 15 minutes
3. 30 minutes
4. 45 minutes
5. 1 hour
6. 1½ hours
7. 2 hours
8. 3 hours
9. 3½ hours
10. 18 hours
11. 20 hours

The die from the impression poured immediately was accurate. The die from the impression stored in water 15 minutes before pouring was undersize. New material was used and another impression made and placed in water 15

minutes before pouring. The die so produced was again undersize. The dies produced from impressions stored in water before pouring 30 minutes, 45 minutes, 1 hour, 1½ hours, and 2 hours, respectively, were accurate. The dies produced from impressions stored in water before pouring for 3 hours and 3½ hours were both undersize; the dies produced from impressions stored in water 18 and 20 hours respectively before pouring appeared to be accurate (Table VI).

TABLE VI
MATERIAL H

TIME INTERVAL BETWEEN MAKING IMPRESSION AND POURING	IMPRESSION PLACED IN:	DIE RESULTS IMPRESSIONS*:	
		NO. 1	NO. 2
Immediate	Water	0	0
15 minutes	Water	–	–
30 minutes	Water	0	0
45 minutes	Water	0	0
1 hour	Water	0	0
1½ hours	Water	0	
2 hours	Water	0	
3 hours	Water	–	0
3½ hours	Water	–	0
18 hours	Water	0	
20½ hours	Water	0	

*Accurate die = 0.
Oversize die = +.
Undersize die = –.

DISCUSSION

In the tests reported in this series of three papers concerning the maintenance of equilibrium in reversible hydrocolloid impression materials, it has become evident that the reversible hydrocolloids fall into two classes: (1) Those materials which over a period of time contract and produce oversized dies after the critical point in the maintenance of their equilibrium is reached, and (2) those materials which expand or swell producing undersized dies after the critical point is reached. Since all these materials are primarily agar bases, it is assumed that this difference in reaction to water or salt solutions must lie in the character of the filler used to add body to the impression material. Seven stock materials purchased in the open market were tested. Four materials supplied by manufacturers were tested. These appeared to act identically with open market materials of the same brand. Four are in the group which tends to contract at the critical point, and 4 in the group which tends to expand or swell at the critical point.

TABLE VII
MATERIALS WHICH:

CONTRACT	EXPAND
A,* B,† D, F	C,* E, G, H

*Maintenance of Equilibrium in Reversible Hydrocolloid Impressions. I. Made With Stock Impression Materials, *J. D. Res.* **28**: 108, 1949.

†Maintenance of Equilibrium in Reversible Hydrocolloid Impressions. II. Made With Materials Supplied by the Manufacturers for Testing, *J. D. Res.* **28**: 119, 1949.

This points to the need of further research into filler materials used in conjunction with agar to produce the commercial reversible hydrocolloid impression compounds.

It is essential to keep in view the fact that with straight-sided perforated trays, such as have been used in this series of tests, the contracting materials will produce oversized dies and the expanding, or swelling materials, will produce undersized dies.

Any material which will not produce accurate dies when poured immediately in impressions made with the material cannot be considered to afford a satisfactory degree of accuracy. Unless this accuracy is obtainable at the beginning of a time period, it is extremely doubtful if changes in volume at any specified time interval thereafter could be relied upon for accurate procedure. No consistent pattern of change appears in those materials which were inaccurate when models are poured immediately.

The question of incompatability between certain hydrocals and certain impression materials has arisen and should be investigated. Nevertheless, it must be noted that not once in the long series reported in Parts I, II, and III of this study of dies poured in the reversible hydrocolloid impressions was there any instance of rough or powdery stone surface. As has been previously stated, tap water was used throughout in these experiments (except for making 2 per cent potassium sulfate solution which was made with distilled water) because the tap water, I believe, more nearly represents conditions under which the materials are used throughout the country. For purposes of record the water analysis has been checked with the Beverly Hills Water Department and the municipal water supply is reported to contain salts by which water is rated for hardness in the range of 7.6 to 8.7 grains per gallon. Since the isotonic solution recommended for one of the impression materials requires 1,440 grains of potassium sulfate per gallon, it is obvious that the salt content of the tap water is of negligible proportions.

SUMMARY

1. In this series of papers, tests following standard procedures have been made with 7 stock reversible hydrocolloid impression materials, and 4 materials supplied by manufacturers, representing 8 different brands.

2. Reversible hydrocolloid impression materials must produce accurate control dies when impressions are poured immediately to be considered acceptable.

3. Reasonable latitude of time between making the impression and pouring the model for accuracy is desirable, but the manufacturer should state specifically the time limit such accuracy may safely be maintained.

4. The materials by brands divide equally into a group which tends to contract at a critical point and a group which tends to expand at a critical point.

5. After the critical point is reached, the contracting group produces oversized dies. The expanding group produces undersized dies.

6. Since natural agar is the base for all reversible hydrocolloid impression materials, a study of fillers used which may cause expansion or contraction is indicated.

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COPPER PLATING OF REVERSIBLE HYDROCOLLOID IMPRESSIONS

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INTRODUCTION

THE accuracy of reversible hydrocolloid for impression materials in indirect inlay work has never been equalled, but heretofore no die material harder than artificial stone has been available for use with these materials. The disadvantage of fragile cavity margins in the poured die is well known.

Blocking off the surface to be plated has been extremely difficult because of the high water content of the hydrocolloid, and the behavior of this material in electroplating is entirely different from that in plating wax. The first coating of the impression must be accomplished before the critical point in the maintenance of equilibrium of the impression material is reached.

The objective of this report is to describe a method of obtaining a metal die accurately reproducing all of the fine detail in a hydrocolloid impression. This method of electroplating a hydrocolloid impression was developed in collaboration with Mr. Harry Rubin, a chemist familiar with electroplating methods.

MATERIALS AND METHODS

This method of electroplating differs from the usual in three respects: displacement of iron by copper; vigorous agitation of the solution during plating; and a special electrolyte. The initial coat of copper must be deposited in three minutes or less so that the electrolyte does not affect the equilibrium of the hydrocolloid. Because no metallic tray of any description may be put into the plating bath, the impression must be removed from the metallic tray before fastening on the plating platform with sticky wax. To prevent distortion in impressions of the entire arch, an inner tray (Fig. 1) of $\frac{1}{64}$ inch Vinylite sheet resin is fitted inside the metal impression tray and is well perforated with $\frac{1}{4}$ inch holes which serve as retention for the hydrocolloid as well as to facilitate thermal conductivity. After removal from the metal tray, this resin tray gives adequate support to the hydrocolloid impression after mounting on the platform for plating.

Method of Plating.—The impression to be plated is immersed in the electrolyte solution (preferably in a separate container to avoid possible contamination of the main bath) for two minutes, then removed, and the excess electrolyte blown off with compressed air. Fine iron powder is immediately sprayed on the area to be plated with a fine jet air gun. The copper ions in the copperplating solution (electrolyte), absorbed by the hydrocolloid during this two-minute immersion, displace the iron ions in the iron powder to form a fine coating of copper on the surface of the impression. The impression is

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again immersed in the electrolyte for two minutes to displace the iron completely, and to form a surface "interlacing" of copper which affords perfect conductivity for the current to be applied. Any excess precipitation may be washed off under the cold-water faucet.

Electric contact is made in the usual manner by enameled copper wire (26 gauge has been found adequate and does not mar the impression unnecessarily) with an enamel-free end imbedded 1 mm. into the hydrocolloid. Two wire contacts have been found adequate for a full arch impression; smaller impressions rarely require more than one. The contact wire may be inserted into the impression either before or after the preliminary coating at a point which will not mar any essential part of the impression (Fig. 2).

Fig. 1.—Impression tray with inner tray.

The electrolyte is agitated by an electrical stirring apparatus. This agitation has been found absolutely essential in rapid plating to prevent burning of the copper. It also is absolutely essential that all immersed metal parts of the agitating device must be insulated by an acid-proof inert plastic coating or paint, of which there are several types available in the market.

One hundred milliamperes are applied for each tooth area to be plated for a period of five minutes. The impression is removed from the bath, and examined for a complete coverage with copper at this time. If there is coverage the current is doubled to 200 milliamperes for each tooth area to completion of the process which may be accomplished in three hours. If the coverage should be incomplete, the electrolyte is blown off with compressed air and iron powder reapplied to the uncovered area before replacing in the electrolyte for completion at the increased amperage.

Since it may require up to 5 amperes for large, full arch impressions, the copperplating machines available to the dental profession at the present time are totally inadequate and could only be used in plating one or two single inlay impressions.

Plating interproximal preparations may best be accomplished by spraying the iron powder on the prepared tooth only so that the plating is confined. Since copper contact points are not essential, stone may be poured into the approximating teeth for contact point purposes, as stone is more easily trimmed than copper. Because the stone may not be placed in the plating bath it is necessary before plating is attempted to inject the approximating teeth full of hydrocolloid from a small syringe, otherwise the thin septum between the proximal cavity margins and the adjacent teeth will allow "creeping" of the copper through the thin wall and a rough margin will result. When the plating is

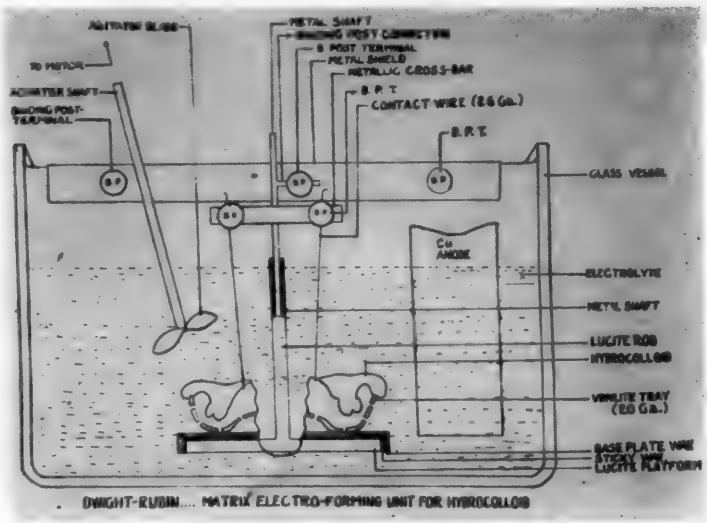

Fig. 2.—Electro-forming unit.

completed these injected areas may be freed of the added hydrocolloid with compressed air and the impression poured with stone. A thin area in the impression between the prepared tooth and the impression tray must be avoided as there is a tendency for the copper to plate on through the thin walls of the impression material instead of stopping at the surface, hence adequate wax stops must be placed in the tray before taking impression.

Determining the surface hardness of plated copper backed with artificial stone has presented quite a problem, since the two approximating materials have entirely different properties and yet are actually combined in copper dies. The backing strength of stone is generally known, so the surface hardness of the copper is our principal concern. The following chart illustrates the advantage in hardness gained by using the addition agent in the electrolyte.

RELATIVE HARDNESS TABLE

The copper with addition agent	Rockwell F Scale 86
Copper without addition agent	Rockwell F Scale 72

(Wrought Copper 72 to 75)

DISCUSSION

Previous attempts to electroplate reversible hydrocolloid impression materials were unsuccessful until the displacement principle of iron-sensitizing powder by copper was developed, the difference in position of the two elements in the electrochemical series is the reason for this displacement, that is, copper displaces iron, this displacement method produces immediate conductivity on the surface to be plated, and the application of electric current thereafter effects extremely rapid coverage.

While this method has been used chiefly in the production of individual indirect inlays, it is applicable to full mouth reconstruction work and the construction of partial dentures. Impressions for the latter purposes require a heavier duty machine than has been marketed heretofore.

SUMMARY AND CONCLUSIONS

1. Through the displacement of iron-positive ions in iron powder sprayed into the impression, by copper-positive ions in the electrolyte solution absorbed by the hydrocolloid during two minutes' immersion, a surface plating of metallic copper is obtained for immediate electrical conductivity.

2. Direct current up to 5 amperes and vigorous agitation of the electrolyte are essential to successful plating of reversible hydrocolloids.

3. The rapid coverage of the impression with copperplate (in less than 15 minutes) insures complete accuracy, since the surface is formed before the equilibrium of the hydrocolloid is affected.

4. The ability to obtain a hard metallic surface die is an important step in developing better models from reversible hydrocolloid impressions.

TOBACCO AND GINGIVITIS

II. Correlation Between Consumption of Tobacco, Ulceromembranous Gingivitis and Calculus

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INTRODUCTION

ON the basis of examinations of Danish Royal Marines, a relation has been demonstrated to exist between (1) consumption of tobacco and ulceromembranous gingivitis and (2) between consumption of tobacco and deposition of calculus.[2] The object of the present work was to study on the basis of twice the quantity of material whether the relation between consumption of tobacco and ulceromembranous gingivitis can be explained by the correlation between consumption of tobacco and deposition of calculus, or whether it is possible to demonstrate an effect of the tobacco per se.

MATERIAL AND METHODS

The material comprised 5,690 Danish Royal Marines who have all been examined by the author, personally. As in the previous paper[2] the gingivae were classified into: normal gingivae, chronic simple marginal gingivitis, and ulceromembranous gingivitis. A distinction was made between nonsmokers, individuals with a consumption of tobacco less than 10 gr. per day, and those with more than 10 gr. per day. The diagnoses of calculus were grouped as no calculus, supragingival calculus, and/or subgingival calculus. Cases of subgingival calculus associated with supragingival calculus were registered in the group subgingival calculus. In the statistical compilation of the material x^2, – values have been calculated according to Fischer.[1] The probability values, P, correspond to these values.

RESULTS

Table I shows the results found on the relation between consumption of tobacco and gingivitis. The number of normal gingivae diminishes with increasing consumption of tobacco and the number of ulceromembranous gingivites rises. Simple chronic marginal gingivitis is not affected by consumption of tobacco. The x^2 value calculated for Table I is 55.1, to which a $P<0.1$ per cent corresponds, indicating that the relation between ulceromembranous gingivitis and consumption of tobacco is significant. The influence of the consumption of tobacco on the deposition of calculus is shown in Table II. The number of cases without calculus declines from 50.0 per cent in nonsmokers, to 30.9 per cent in those smoking more than 10 gr. tobacco per day. Conversely, there is an increase in the percentage of supragingival as well as subgingival calculus with a rising consumption of tobacco. The increase is greatest in the case of the supragingival calculus. For Table II, $x^2 = 83.5$. The $P<0.1$ per cent. This is significant.

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TABLE I

SHOWING CORRELATION BETWEEN CONSUMPTION OF TOBACCO AND INCIDENCE OF GINGIVITIS

	NONSMOKERS		< 10 GR.		≧ 10 GR.		TOTAL	
	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
Normal gingiva	190	33.5	508	28.3	785	23.6	1,483	26.0
Chronic simple marginal gingivitis	367	64.6	1,175	65.3	2,258	67.9	3,800	66.8
Ulceromembranous gingivitis	11	1.9	115	6.4	281	8.5	407	7.2
Total	568	100.0	1,798	100.0	3,324	100.0	5,690	100.0

TABLE II

SHOWING CORRELATION BETWEEN CONSUMPTION OF TOBACCO AND INCIDENCE OF CALCULUS

	NONSMOKERS		< 10 GR.		≧ 10 GR.		TOTAL	
	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
Minus calculus	284	50.0	606	33.7	1,028	30.9	1,918	33.8
Supragingival calculus	236	41.5	983	54.7	1,871	56.3	3,090	54.2
Subgingival calculus	48	8.5	209	11.6	425	12.8	682	12.0
Total	568	100.0	1,798	100.0	3,324	100.0	5,690	100.0

Furthermore, in the same material, a study has been made on the relation between calculus and gingivitis. Table III shows, as was indeed to be expected, that the number of normal gingivae is largest among those who are calculus free. Among those in whom supragingival calculus is present, only 13.8 per cent have normal gingivae, while 76.9 per cent and 9.3 per cent, respectively, present simple marginal gingivitis and ulceromembranous gingivitis. A study of the figures of the subgingival calculus gives the result that this form of calculus is by far the most harmful to the gingival tissues. $\chi^2 = 1182.6$, $P<01$ per cent, and the figures are significant.

TABLE III

SHOWING CORRELATION BETWEEN INCIDENCE OF CALCULUS AND GINGIVITIS

	MINUS CALCULUS		SUPRAGINGIVAL CALCULUS		SUBGINGIVAL CALCULUS		TOTAL	
	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
Normal gingiva	1,027	53.6	426	13.8	30	4.4	1,483	26.1
Chronic simple marginal gingivitis	848	44.2	2,376	76.9	576	84.5	3,800	66.8
Ulceromembranous gingivitis	43	2.2	288	9.3	76	11.1	407	7.1
Total	1,918	100.0	3,090	100.0	682	100.0	5,690	100.0

Since a correlation has now been demonstrated between consumption of tobacco and gingivitis, between consumption of tobacco and deposition of calculus, and finally between calculus and gingivitis, it is very natural to study whether the dependence between gingivitis and consumption of tobacco may be explained by the relation between consumption of tobacco and calculus. In order to ascertain whether this be the case, an examination must be carried out within each group to show whether there exists any dependence between consumption of tobacco and gingivitis. Such a study has been carried out on part

of the material (3,505 Marines) and the results are presented in Table IV. It demonstrates that the incidence of ulceromembranous gingivitis rises with increasing consumption of tobacco within all three groups. In the group minus calculus, the incidence rises from 1.3 per cent in nonsmokers, to 3.9 per cent in moderate smokers, and to 4.2 per cent in the heaviest smokers. In the case of supragingival calculus, the rise is from 2.2 per cent to 8.7 per cent and to 11.9 per cent and, finally, in the group: subgingival calculus, 6.7 per cent to 8.8 per cent and to 12.4 per cent. The χ^2 values calculated are 4.3, 25.9, and 2.1, to which P values between 30 and 80<0.1 and 30 – 80 per cent correspond. The total χ^2 for the three tables thus is 32.3 (P<0.1 per cent). This means that, unless the view is maintained that special factors are at work in the case of supragingival calculus, that the dependance between consumption of tobacco and gingivitis is also in evidence after the classification according to the presence of calculus, but that the material of the calculus-free gingivae and subgingival calculus is too small to show the difference statistically. The total χ^2 shows, however, that the χ^2 corresponding to supragingival calculus cannot be presumed to have become significant for accidental reasons, since the probability hereof continues to be less than 0.1 per cent. In other words, in addition to the effect caused by the increased deposition of calculus, the tobacco has an effect, per se, on the gingival tissues so as to make these more susceptible to ulceromembranous gingivitis.

TABLE IV

SHOWING CORRELATION BETWEEN CONSUMPTION OF TOBACCO AND GINGIVITIS WITHIN EACH OF THE THREE GROUPS OF CALCULUS

A: Minus Calculus

	NONSMOKERS		< 10 GR.		≧ 10 GR.		TOTAL	
	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
Normal gingiva	95	60.1	193	53.5	301	54.8	589	55.1
Simple gingivitis	61	38.6	154	42.6	225	41.0	440	41.2
Ulceromembranous gingivitis	2	1.3	14	3.9	23	4.2	39	3.7
Total	158	100.0	361	100.0	549	100.0	1,068	100.0

B: Supragingival Calculus

	NONSMOKERS		< 10 GR.		≧ 10 GR.		TOTAL	
	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
Normal gingiva	24	17.4	110	20.9	123	13.5	257	16.3
Simple gingivitis	111	80.4	370	70.4	682	74.6	1,163	73.7
Ulceromembranous gingivitis	3	2.2	46	8.7	109	11.9	158	10.0
Total	138	100.0	526	100.0	914	100.0	1,578	100.0

C: Subgingival Calculus

	NONSMOKERS		< 10 GR.		≧ 10 GR.		TOTAL	
	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
Normal gingiva	1	3.3	6	4.4	14	5.4	21	5.0
Simple gingivitis	27	90.0	118	86.8	212	82.2	357	84.2
Ulceromembranous gingivitis	2	6.7	12	8.8	32	12.4	46	10.8
Total	30	100.0	136	100.0	258	100.0	424	100.0

SUMMARY

By examination on 5,690 Danish Royal Marines a correlation has been found between consumption of tobacco and ulceromembranous gingivitis, between consumption of tobacco and deposition of calculus, and between calculus and gingivitis. Furthermore, it is shown, solely by means of statistical data, that the tobacco has an effect, per se, on the gingival tissues.

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TOBACCO AND GINGIVITIS

III. Difference in the Action of Cigarette and Pipe Smoking

ASGER FRANDSEN AND JENS JØRGEN PINDBORG

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INTRODUCTION

PINDBORG[1] has demonstrated positive relationships between consumption of tobacco and ulceromembranous gingivitis between consumption of tobacco and deposition of calculus on the other. In the present study the object has been to establish whether pipe smoking and cigarette smoking have the same effect on these correlations.

MATERIAL AND METHODS

In part of the material, which comprises 5,690 Marines, the use of tobacco was more fully specified, and the Marines grouped as exclusively cigarette smokers or exclusively pipe smokers, and smokers of cigarettes and pipes. In the compilation of the material for this particular purpose, only those Marines were included who were exclusively either cigarette smokers or pipe smokers. The variations involved are of such a magnitude that a further subdivision into smokers of cigarettes and pipes, with a preponderance of either one or the other of the two forms, would be quite illusory; 1,848 were exclusively cigarette smokers and 273 exclusively pipe smokers. For the purpose of ascertaining possible differences t and χ^2 tests[2] have been utilized.

RESULTS

Table I shows a correlation of the form of smoking with the condition of the gingivae. If a comparison is made of the number of normal gingivae in the two groups, it is found that $t = 1$, and for ulceromembranous gingivitis $t = 1.2$. Both are insignificant. The whole table gives $\chi^2 = 3.1$, corresponding to probabilities 30 per cent $< P <$ 20 per cent, which is also insignificant. Thus, no difference has been established in the effects of cigarette and pipe smoking on the gingival tissues.

Table I

Showing Mode of Smoking Correlated With Clinical Gingival Diagnosis

Diagnosis	Mode of Smoking					
	Cigarette Smokers		Pipe Smokers		Total	
	Number	%	Number	%	Number	%
Normal gingiva	426	23.1	55	20.1	481	22.7
Chronic simple marginal gingivitis	1,305	70.6	206	75.5	1,511	71.2
Ulceromembranous gingivitis	117	6.3	12	4.4	129	6.1
Total	1,848	100.0	273	100.0	2,121	100.0

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An interesting difference in the effects of cigarette and pipe smoking on calculus formation is in evidence. Table II shows the results of the study. A t calculated for those without calculus is equal to 4.3, which is significant. Calculated for subgingival calculus it is found to be 1.2, which is insignificant. For the whole table $x^2 = 18.6$, corresponding to $P<0.1$ per cent, indicating that there is a significant difference between cigarette and pipe smokers.

TABLE II

SHOWING MODE OF SMOKING CORRELATED WITH INCIDENCE OF CALCULUS

	MODE OF SMOKING					
	CIGARETTE SMOKERS		PIPE SMOKERS		TOTAL	
CALCULUS	NUMBER	%	NUMBER	%	NUMBER	%
Minus calculus	566	30.6	49	17.9	615	29.0
Supragingival calculus	1,065	57.7	185	67.8	1,250	58.9
Subgingival calculus	217	11.7	39	14.3	256	12.1
Total	1,848	100.0	273	100.0	2,121	100.0

The chances of the deposition of supragingival calculus is thus greater for pipe smokers than for those smoking cigarettes only. The explanation of the difference in action is not yet known. It would, however, be natural to assume that in pipe smoking there is a stronger salivation than in cigarette smoking, and that, consequently, more material is available for the deposition of calculus.

SUMMARY

The presence of gingivitis and calculus has been studied in 1,848 Marines smoking cigarettes only and 273 smoking pipes only. While there is no difference as regards the incidence of gingivitis, a significantly greater deposit of calculus is observed in pipe smokers than in cigarette smokers.

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SOME CHARACTERISTICS OF CARIES ON THE PROXIMAL SURFACES OF THE TEETH

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SOME characteristics of caries have been revealed by study of the incidence of attack on over 20,000 proximal tooth surfaces. The investigation (Table I) covered over 500 cases, ranging in age from five to thirty years, presenting wide variations in evidence of caries activity. The information utilized in this report is based on both physical (clinical) and radiographic examination of the teeth, the diagnosis of proximal caries experience being therefore as reliable as possible. Earlier reports with specific reference to *proximal* caries are not numerous; of these, most appear to be based on clinical examination alone, with a consequent undesirable distortion of the true caries picture. In its first part, this study is concerned with the general characteristics of bilateral symmetry of proximal caries experience, regional correlation, and the factors of age, sex, and approximation of surfaces. In the second part, more specific information is presented concerning the sequence in which caries tends to originate on proximal tooth surfaces, that is, the relative susceptibility of these surfaces.

TABLE I
SUMMARY OF GROUPS EXAMINED

IDENTIFICATION LETTERS OF GROUP	AGES (AT LAST BIRTHDAY)		NUMBER OF CASES IN GROUP			PROXIMAL SURFACES EXAMINED			PROXIMAL SURFACES CARIOUS (C.R.L.)		
	RANGE	MEDIAN	M	F	TOTAL	ANTERIOR	POSTERIOR	TOTAL	ANTERIOR	POSTERIOR	TOTAL
X	5-14	7.5	101	93	194		4656	4656		1907	1907
T	13	13.5	30	30	60	1200	1440	2640	263	624	887
M	17-35	20.0	150	30	180	3600	5760	9360	1166	3659	4825
A	17-30	22.5	47	33	80	1600	2560	4160	187	1212	1399
					514	6400	14416	20816	1616	7402	9018

GENERAL CHARACTERISTICS

1. Bilateral Symmetry

The frequency with which proximal caries occurred has been first examined for evidence of bilateral symmetry (a) in total caries experience for whole groups (Table II a), and (b) in group totals by individual tooth surfaces (Fig. 1). It has seemed logical for purposes of this paper to regard division into anterior and posterior regions of the mouth as being located through the center of the canine tooth in each quadrant, the distal surface of this tooth being included with posterior proximal surfaces. Proximal caries has next been studied for correlation between its total occurrence in corresponding regions of right and left sides of individuals (Fig. 2). Finally the actual occurrence of bilateral symmetry of proximal caries has been examined in individuals for the detail of its pattern of distribution on tooth surfaces (Tables II b, II c).

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This material reveals high correlation between proximal caries experience of the teeth on right and left sides of the mouth, extending as a collective or group phenomenon down through regional subdivisions to individual tooth surfaces. This evidence of generalized close bilateral symmetry of caries attack justifies the policy adopted throughout the remainder of this study, namely that of bilateral combination of all grouped figures. At the same time, it should be noted that bilateral symmetry, while appearing as a strong tendency displayed in individuals when studied by their separate tooth surfaces, was seldom achieved as a completed individual oral pattern (Table II b). In the case of any one individual, the assumption of bilateral symmetry may not therefore be applied too literally; it was shown rather as an aggregate characteristic.

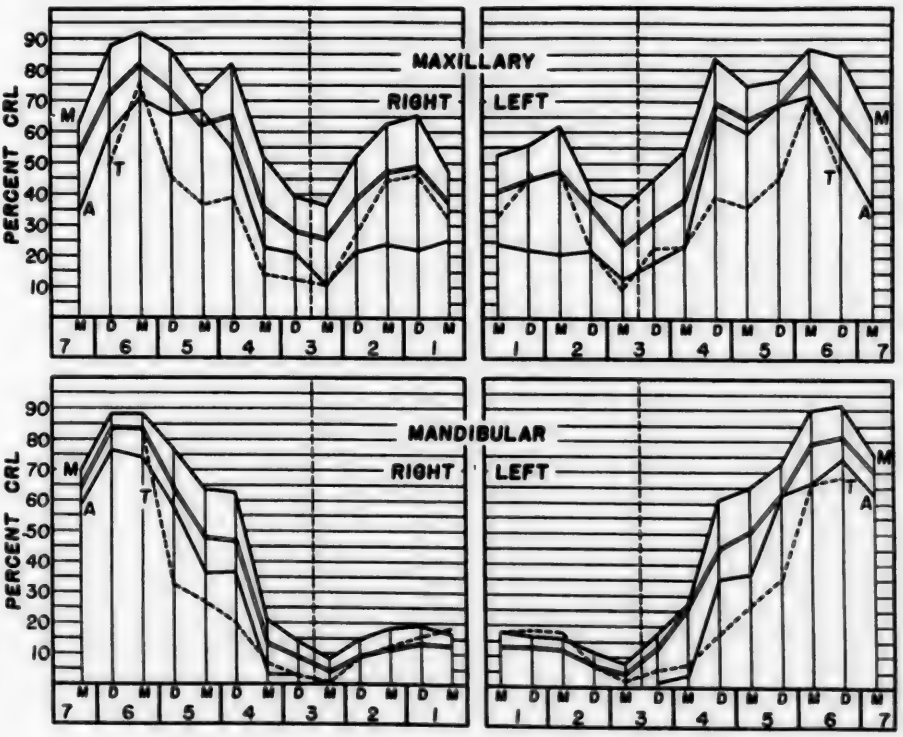


Fig. 1.—General bilateral symmetry of proximal caries. Percentages of surfaces found C.R.L. (carious, restored by fixed restorations, or lost through caries). Series T, M, and A; 60, 162, and 80 cases respectively. Also means for combined figures for these 302 cases (double line). From unpublished data.

2. Interregional Correlation

The association between the incidence of caries attacks on proximal surfaces in different regions of the mouth is presented in Fig. 3. From Fig. 3, *a* it may be seen that proximal caries incidence in maxillary and mandibular posterior regions displayed close similarity, the frequency of carious attack varying together in these regions throughout the series studied.

TABLE II

BILATERAL SYMMETRY OF CARIOUS ATTACK ON THE PROXIMAL SURFACES OF TEETH

(a) GROUPED TOTALS FOR CARIOUS LESIONS

	RIGHT SIDE	LEFT SIDE
Anterior maxillary region*	584	613
Anterior mandibular region*	154	158
Posterior maxillary region	1755	1744
Posterior mandibular region	1550	1551
Total, 424 cases, series TMAX	4043	4066

(b) PATTERNS OF CARIOUS ATTACK IN INDIVIDUALS

Complete bilateral symmetry	5 cases (including 2 cases completely free from caries)
Bilateral symmetry by regions:	
Anterior maxillary region	18 cases
Posterior maxillary region	10 cases
Posterior mandibular region	16 cases
(80 cases, series A)	

(c) CARIOUS ATTACK ON SPECIFIC PROXIMAL SURFACES IN INDIVIDUALS
(Percentages of cases in which bilateral symmetry of attack occurred)

ANTERIOR MAXILLARY		POSTERIOR MAXILLARY		POSTERIOR MANDIBULAR	
1 mesial	65.0%	3 distal	43.7%	3 distal	--
1 distal	55.6%	4 mesial	56.4%	4 mesial	--
2 mesial	55.6%	4 distal	78.5%	4 distal	55.2%
2 distal	47.0%	5 mesial	78.5%	5 mesial	53.6%
3 mesial	22.5%	5 distal	82.4%	5 distal	81.2%
		6 mesial	86.3%	6 mesial	86.8%
		6 distal	70.4%	6 distal	98.3%
(80 cases, series A)		7 mesial	65.7%	7 mesial	95.8%

*Excluding 122 cases, series X.

There was likewise, in those cases where caries affected maxillary anterior teeth, substantially parallel variation in incidence between that region and the two regions referred to above, which together comprise the proximal surfaces of all posterior teeth (Fig. 3, *b*). But an interesting feature was revealed, of which no previous report has been found. In thirty-eight of one hundred seventeen cases, all of which in common presented intact dentitions with all teeth approximated, posterior proximal caries occurred, extending over the whole range of severity of attack, in the absence of any maxillary anterior caries proximally. There is no obvious explanation for this immunity in the anterior region, occurring in approximately one-third of the cases and unrelated to varying degrees of posterior attack, while the other two-thirds displayed well-correlated attack throughout maxillary anterior and posterior regions. A comparable measure of correlation did not extend to the remaining region, the mandibular anterior teeth (Fig. 3, *d*).

Complete caries immunity of mandibular anterior proximal surfaces was likewise evident side by side with varying attack in posterior regions, in this instance in a substantial majority of cases. (Series A, 78 of 80 cases; Series M, 114 of 162 cases; and Series T, 45 of 60 cases.) But there was no indication of any significant interregional correlation in the smaller number of cases where proximal caries was instanced in the mandibular anterior teeth (Fig. 3, *c*).

3. Approximation of Surfaces

Proximal caries has been commonly associated with the factor of approximation of mesial or distal surfaces of adjacent teeth; this is well supported by the current investigation (Fig. 4, *a*). The percentages of surfaces recorded

as carious have likely been affected in some cases by either recent approximation or recent spacing of surfaces through drifting of teeth in incomplete dentitions. Some of the surfaces approximated at the time of mouth examination probably had in reality an earlier history of being spaced, and vice versa in the case of surfaces spaced at the time of examination. The resultant influences on the caries pictures may not be viewed as necessarily self-cancelling, but the wide disparity between caries experience of approximated and spaced surfaces leaves no room for doubt in interpretation. The sharply increased susceptibility of

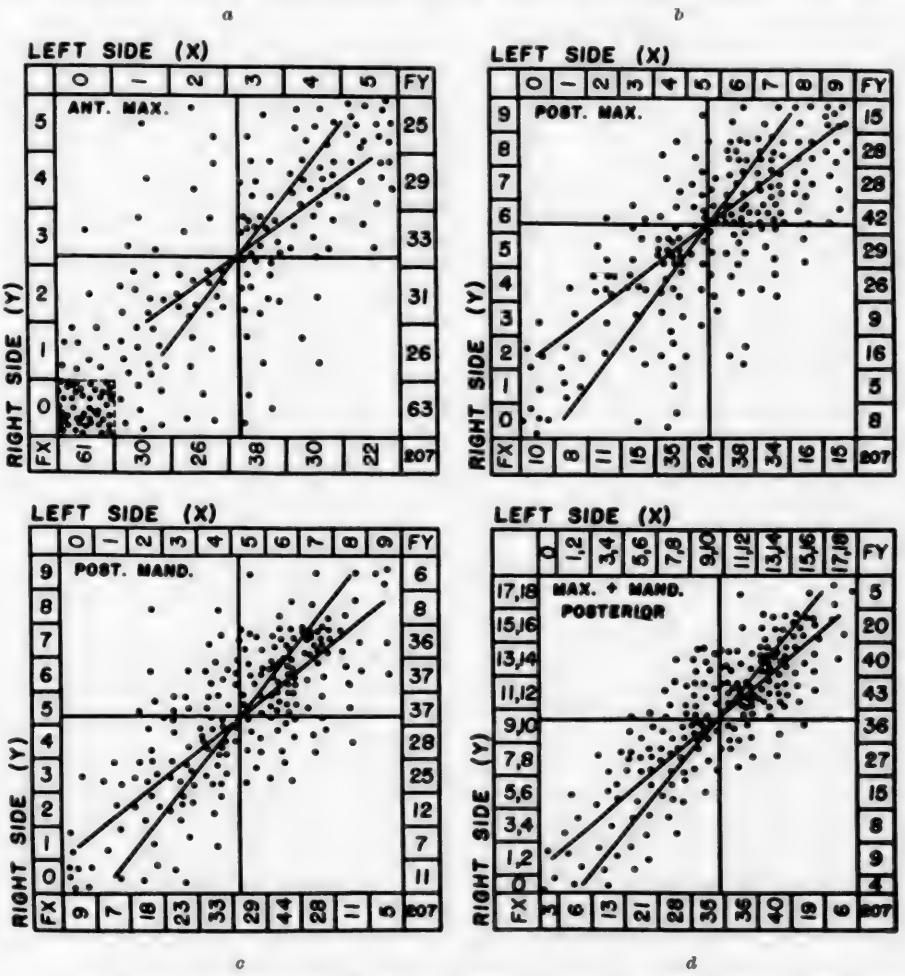


Fig. 2.—Bilateral symmetry of proximal caries. Scatter diagrams showing high positive correlation between regional totals of proximal surfaces C.R.L. for right and for left sides in individual cases. Positive correlations 0.75 or better calculated from measurement of regression lines on the originals of the scatter diagrams reproduced here, and checked by computation in one case (*d*) indicating a correlation in that instance of plus 0.81. (Several of the correlation diagrams presented in this paper have also been checked by conversion of the data to standard measures.) Series M and A, 207 cases, grouped as in Table III.

a, Anterior maxillary region.
b, Posterior maxillary region.
c, Posterior mandibular region.
d, Posterior maxillary and mandibular regions together.

approximated surfaces is further borne out by examination of the frequency of caries experience in the form of attacks simultaneously involving pairs of approximated adjacent surfaces (Fig. 4, *b*).

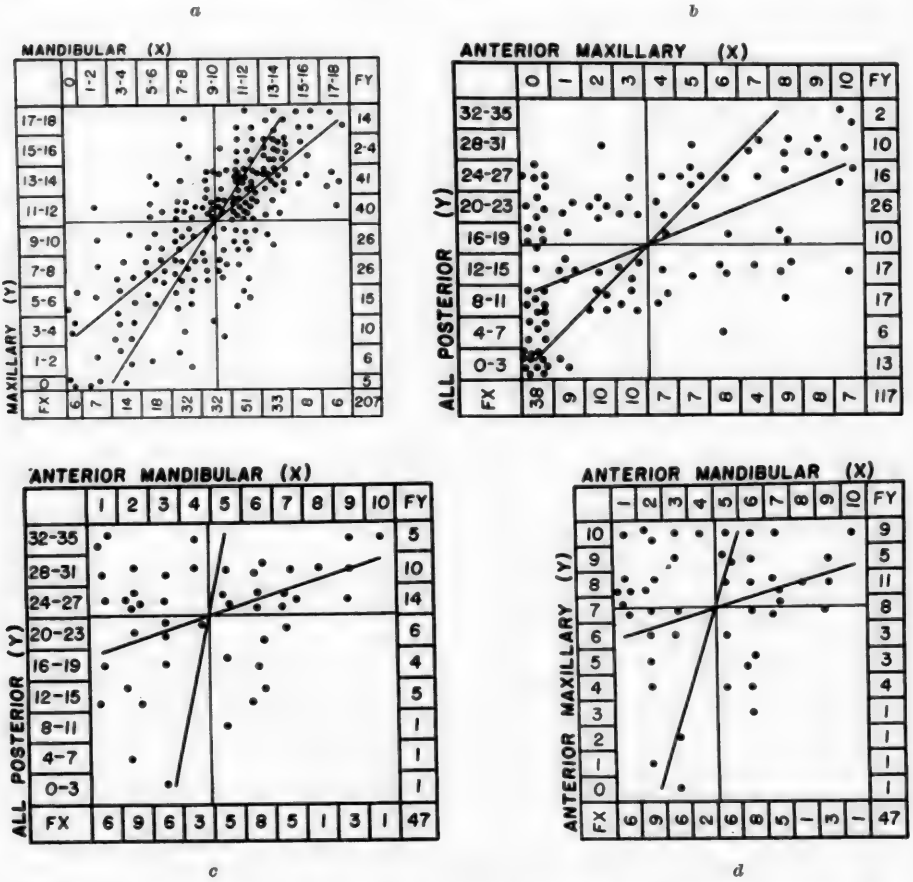


Fig. 3.—Interregional correlations for proximal caries. Scatter diagrams showing correlations between regional totals of surfaces C.R.L. in individual cases.

a, Posterior maxillary region and posterior mandibular region. Positive correlation 0.7. Compiled from the mouth examination charts of 207 cases, Series M and A, as in Table III.

b, Posterior regions and anterior maxillary region. In those cases in which proximal caries occurred in both maxillary anterior teeth and in the posterior teeth, a positive correlation of 0.65 was found; but in cases where there was an absence of proximal caries in maxillary anterior teeth, there was no correlation in the form of a corresponding absence or even a uniformly low figure for posterior surfaces C.R.L. Compiled from the mouth examination charts of 117 complete dentitions, Series T. M., and A, as in Table III.

c, Posterior regions and anterior mandibular region. Low positive correlation of 0.3. Compiled from the mouth examination charts of the 47 cases in which there were carious proximal surfaces in the mandibular anterior region.

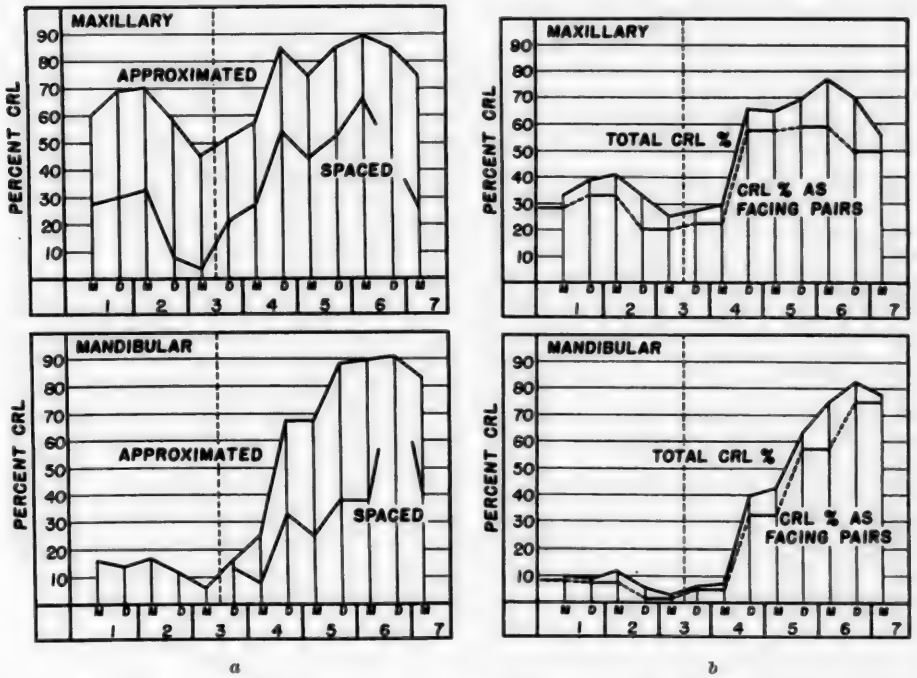
d, Anterior maxillary region and anterior mandibular region. Again a low positive correlation, 0.3. Same 47 cases as in (*c*).

4. Age and Sex

The survey covered groups which proved unsuitable in their age composition to provide a clear picture of continuing caries increments by age save in the group of children whose posterior teeth have been studied through age levels of the mixed dentition. For this group the numbers of proximal surfaces

carious per patient in posterior teeth (including permanent first molars), from 5 years through 8 years, were as follows:

5 years	7.6
6 years	9.0
7 years	12.2
8 years	19.0



a b

Fig. 4.—Proximal caries experience related to approximation of surfaces.

a, Profile representation comparing percentages of approximated and of spaced proximal tooth surfaces attacked by caries. Prepared from an unpublished table, Series M, 162 cases.

b, Proportion of proximal decays in complete dentitions occurring as pairs facing one another on approximated tooth surfaces. Prepared from an unpublished table, Series TMA, 117 cases.

Despite published reports by other workers indicating sexual differentiation in carious attack, the evidence from this study was neither clearly to that effect nor to the contrary. There are instances in which the figures for caries incidence in females should probably undergo downward adjustment before comparisons are made with figures for males, for example, age groups of early adolescence. Due to earlier eruption of teeth in females, the post-eruption age of teeth, and consequently the period of subjection to caries attack, has been greater, giving a proportionate apparent increase in caries experience. Appropriate allowance for this is difficult to attempt. At higher age levels the post-eruption age factor decreases in significance. In most of the age groups examined, the reported incidence in females proved to be slightly higher than in males; but in the largest group (Series M), and consequently also in the figures for all groups combined, it was rather lower in females than in males.

RELATIVE CARIES SUSCEPTIBILITY OF INDIVIDUAL PROXIMAL SURFACES

5. Important Considerations

As an introduction to problems encountered in attempting a detailed evaluation of caries experience surface by surface, Fig. 5 is impressive. It is presented in order to illustrate and to emphasize the extent to which it is possible, through differing methods of assessing caries experience, to introduce variations in the resulting picture. The range of these variations stems chiefly from the manner in which teeth not present in the dentition are handled in the analysis of primary mouth examination data. The direct effect of the misfortune of a lost tooth is the elimination of definite evidence of carious im-

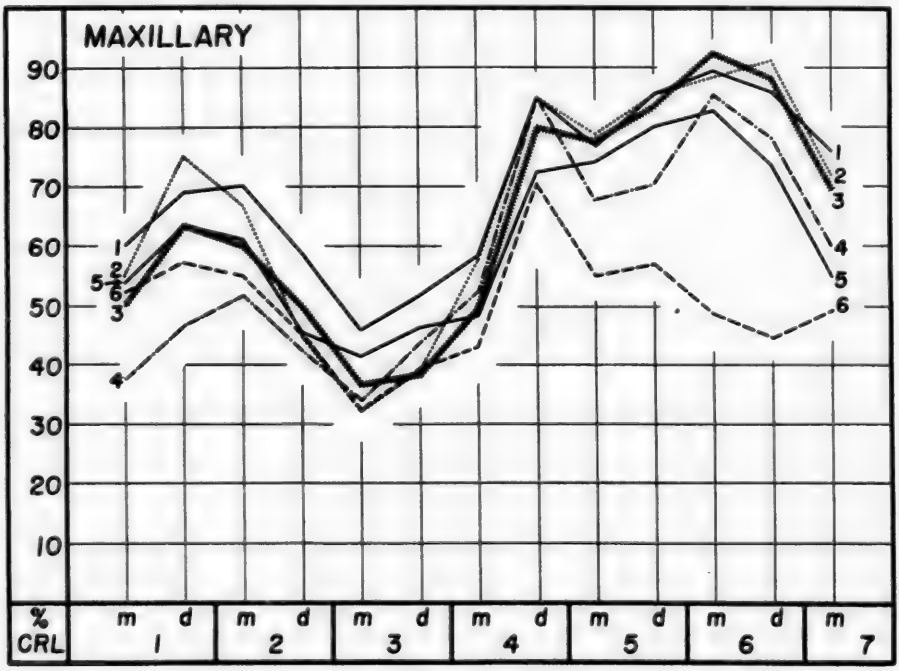


Fig. 5.—Variations in findings resulting from different procedures in analysis of data concerning caries experience of proximal tooth surfaces. Superimposed profiles each representing percentages of individual tooth surfaces treated as carious according to six different procedures for analysis of primary data. Based on unpublished data for maxillary teeth, Series M, 62 complete or intact dentitions and 100 incomplete dentitions, of which 35 were unsuitable for inclusion in C.R.L. data by reason of bilateral loss of teeth, etc. For explanation of C.R.L. analyses, see text.

1, Data from only those surfaces actually in approximation with an adjacent surface.
5, Data from only those complete dentitions in which all teeth were approximated.
3, C.R.L. data from complete and incomplete dentitions considered together.
2, C.R.L. data from incomplete dentitions only.
6, D.F. figures, from incomplete dentitions only, disregarding teeth lost or missing.
4, D.F.M. figures from incomplete dentitions only, assuming all surfaces of teeth missing to have been carious.

munity or attack on its surfaces. Indirectly predisposition to caries, and consequently caries experience, of at least some of the teeth remaining in an incomplete dentition is influenced by modification of the environmental factor of approximation of tooth surfaces. This environmental change occurs by reason

of drifting of teeth once the continuity of a dental arch is broken, and it is not always limited to teeth immediately adjacent to the edentulous space.

These phenomena have not called for special consideration during the study of general characteristics of proximal caries in the earlier part of this paper. Their effect could be expected to show equally, for instance, on right and left sides, without disturbance of the study of bilateral symmetry. Nor would they call for further attention if they operated with equal frequency and with a constant end result in the case of each and every tooth in the dentition. But this not being so, in embarking on a study of the relative caries susceptibility of the separate surfaces of each of the teeth, it becomes necessary to devise a procedure designed to eliminate or at least minimize the distortion which would otherwise be carried through into findings from analysis of primary data. Reference to Figs. 4 and 6 will contribute to an appreciation of the problem raised here.

It would be simpler to eliminate from study all incomplete dentitions. This introduces the principle of selection—in this case a selection of cases of characteristically lower caries susceptibility, for through the age groups with which we are here concerned, the prime cause of incomplete dentitions has been loss of teeth by reason of advanced caries. As one result of such selection, there would be little opportunity to study on a comparative basis the caries experience of surfaces requiring a high level of general caries susceptibility for their attack, that is, to study those surfaces least frequently affected.

An alternative which appears to avoid selection of cases is to use, for purposes of analysis, data from all cases, but only from those tooth surfaces actually in approximation at the time of examination. On consideration this procedure is found to have little more to recommend it than the selection of complete or intact dentitions. While it achieves the objective of excluding misleading data obtained from drifted teeth, etc., it necessarily relates caries experience figures for each surface to a different total number of cases grouped differently in each instance. The different groups so constituted will not prove suitable for purposes of valid comparison.

We are returned to the problem of devising a basis for analysis which should have sufficiently general application and which, recognizing that our original primary data will not necessarily reflect a true measure of the caries susceptibility for each tooth, should provide for compensatory adjustment concerning teeth lost, undeveloped, or unerupted, and especially concerning the consequent change in the "approximational" environment of other teeth in the same dentition.

The procedure developed for analysis of data in this study is not claimed to be ideal. It involves the application of findings discussed earlier—that caries tends to occur in a bilaterally symmetrical pattern, and that the caries incidence is atypical in cases where teeth have become removed from physical approximation with adjacent teeth. Given a knowledge of these general characteristics of caries, a dentition intact on one side, the disposition of existing teeth on a nearly complete opposite side, and the caries findings from examina-

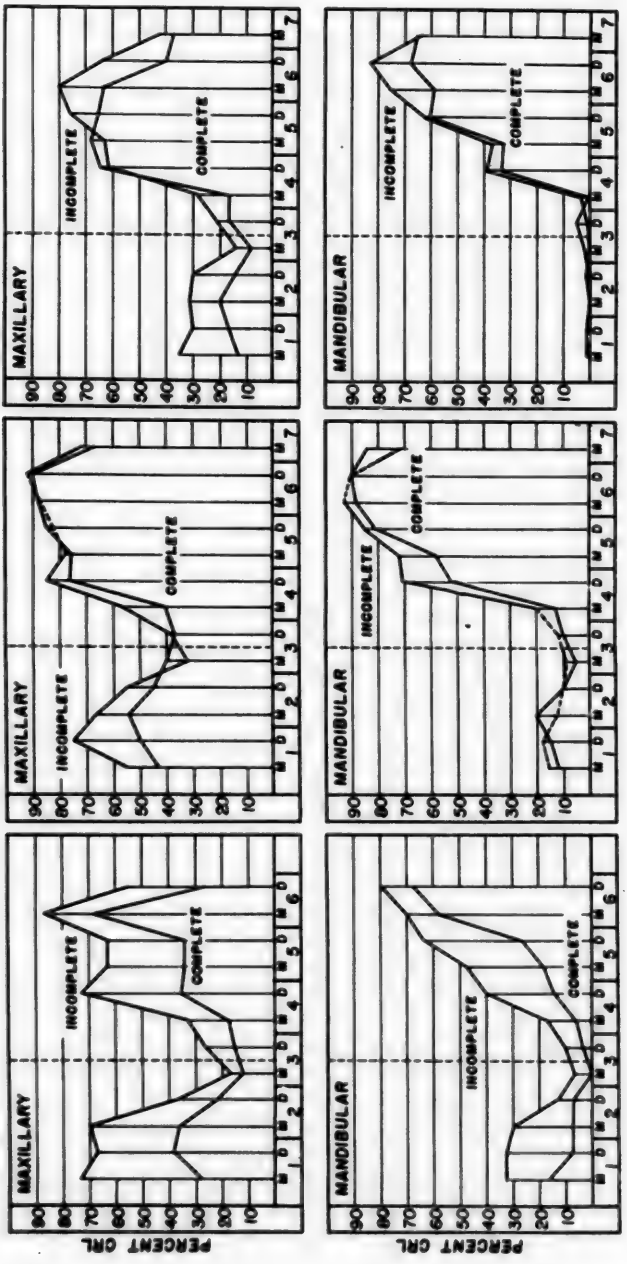


Fig. 6.—Proximal caries patterns in complete and incomplete dentitions. Contrasting profiles compiled from Table III. *a*, Series T. *b*, Series M. *c*, Series A.

tion of both sides, then it should be possible to project or reconstruct, with insignificant opportunity for error, a more probably true caries picture for all teeth on the incomplete side; that is, one more compatible with the situation which would have been encountered had the integrity of the arch on that side not have suffered disturbance. Any consequent included error in the adjusted data holds less potentiality for distortion of the analysis than direct use of the primary data.

This procedure has not entirely removed the need for some selection of cases. There were a few cases in which too many teeth had been lost to offer promise of any highly probable measure of accuracy in the reconstruction of a better caries experience picture for the case by reference to an opposite side of the mouth. Some of the more incomplete or mutilated dentitions therefore required to be eliminated, but the effect of selection has been sharply reduced. It is believed that residual distortion of findings has been minimized and more truly typical group patterns obtained than could be achieved by other methods. The procedure calls for differentiation in name from the widely used D.M.F. counts. The terminology carious, restored (by fixed restoration), or lost (through caries), is therefore introduced, for convenience abbreviated to C.R.L. The use of the term C.R.L. will be understood to embrace a measure of adjustment of primary data in cases of incomplete dentitions, designed to compensate for lost or missing teeth and the associated sequelae. (The term C.R. will serve in the case of intact or complete dentitions and in such cases will obviously be identical with a D.F. count.)

No C.R.L. adjustment of primary data was attempted for the last molar teeth or for the adjacent surface of the second last molar teeth. Caries experience of these teeth in developing or recently matured dentitions can give no real indication of the relative susceptibility of those surfaces. Figures for those surfaces are given only in Table III and appear in parentheses; they are actual findings for only those surfaces actually approximated to another surface and therefore subject to limitations of interpretation previously referred to.

Separate details for complete dentitions with all teeth approximated, included in Table III, will serve for reference should a study of unadjusted primary data for intact dentitions be judged preferable to figures on a C.R.L. basis.

6. Sequence of Origin of Caries on Proximal Surfaces

Two methods may be used for study of the progressive or incremental development of proximal caries attack in groups of cases—(a) study of successive age levels and (b) study at successive levels of intensity of developed attack.

Carious attack was so advanced, even at the low extremities of the age range in Series M and A, that study at successive age levels within these groups proved unsuitable. Neither series lent themselves to successful differentiation into age groups yielding a continuing picture of the extending caries pattern. A gross idea of development of the caries pattern, from data at two more widely separated age levels covering characteristically similar groups, is given in Fig. 7.

TABLE III

CARIES EXPERIENCE (C. R. L.) OF INDIVIDUAL PROXIMAL SURFACES

	SERIES T 40 CASES			SERIES M 127 CASES			SERIES A 80 CASES			SERIES T-M-A 247 CASES
	COMPLETE DENTITIONS	INCOMPLETE DENTITIONS	ALL DENTITIONS	COMPLETE DENTITIONS	INCOMPLETE DENTITIONS	ALL DENTITIONS	COMPLETE DENTITIONS	INCOMPLETE DENTITIONS	ALL DENTITIONS	ALL DENTITIONS
	% C.R.L.	% C.R.L.	% C.R.L.	% C.R.L.	% C.R.L.	% C.R.L.	% C.R.L.	% C.R.L.	% C.R.L.	% C.R.L.
Maxilla										
1 mesial	28.0	73.3	45.0	44.3	54.6	49.6	13.3	35.0	26.8	41.5
1 distal	38.0	66.7	48.8	50.0	75.3	63.0	16.7	30.0	26.8	49.0
2 mesial	36.0	70.0	48.8	54.0	66.1	60.2	20.0	32.0	27.5	47.8
2 distal	22.0	36.7	27.5	45.9	55.4	50.4	15.0	30.0	24.4	38.3
3 mesial	12.0	16.7	13.8	40.3	31.5	35.8	6.7	14.0	11.2	24.3
3 distal	16.0	26.7	20.0	37.9	38.5	38.2	16.7	22.0	20.0	29.4
4 mesial	18.0	33.3	23.8	41.1	56.9	49.2	16.7	29.0	24.4	37.0
4 distal	36.0	73.3	50.0	76.6	84.6	80.8	65.0	62.0	63.2	70.2
5 mesial	34.0	63.3	45.0	75.8	77.7	76.8	68.2	64.0	65.7	68.0
5 distal	34.0	63.3	45.0	84.7	83.1	83.9	65.0	76.0	71.9	73.7
6 mesial	68.0	86.7	75.0	88.0	87.7	91.8	63.3	80.0	73.8	83.2
6 distal	26.0	56.7	42.8	89.7	90.7	87.8	40.0	63.0	54.4	71.0
7 mesial			(8.9)	65.3	71.5	68.5	36.7	40.0	40.0	(51.7)
7 distal						(35.2)			(24.1)	(30.1)
8 mesial						(24.5)			(11.4)	(18.5)
Mandible										
1 mesial	16.0	33.3	22.5	12.0	14.6	13.4	---	1.0	0.6	10.7
1 distal	8.0	33.3	17.5	14.5	16.9	15.7	---	1.0	0.6	11.1
2 mesial	8.0	30.0	16.3	20.2	11.5	15.7	---	---	---	10.7
2 distal	8.0	13.3	10.0	10.5	10.8	10.6	---	---	---	7.3
3 mesial	---	6.7	2.5	4.8	9.2	7.1	---	---	---	4.3
3 distal	4.0	10.0	6.3	10.5	11.5	11.0	5.0	1.0	2.5	7.5
4 mesial	6.0	16.7	10.0	13.7	20.0	16.9	---	3.0	1.9	10.9
4 distal	14.0	40.0	23.8	53.2	70.8	62.2	33.3	39.0	36.9	47.8
5 mesial	18.0	46.7	28.8	58.0	72.2	65.4	31.7	36.0	34.4	49.4
5 distal	26.0	63.3	40.0	81.5	86.2	83.8	60.0	61.0	60.6	69.3
6 mesial	58.0	70.0	62.5	88.0	93.1	90.6	58.3	75.0	68.7	79.0
6 distal	68.0	80.0	72.3	89.5	90.0	89.8	68.3	82.0	76.9	83.2
7 mesial			(39.7)	83.8	72.2	77.9	65.0	63.0	63.8	(68.4)
7 distal						(46.1)			(28.2)	(37.0)
8 mesial						(26.3)			(11.5)	(18.8)

Age groupings do offer a clear picture of the sequence of origin of caries on proximal surfaces in the posterior teeth of children (Series X) through from 5 to 8 years of age (Table IV, Fig. 8). Similar findings for a portion of the same series of cases have been published in an earlier paper by the author, and these are here confirmed and extended. There should be clinical application for the finding, well shown in the illustration, that distal surfaces of the deciduous molar teeth tend to become carious earlier than the mesial surfaces of the adjacent teeth.

Considerably successful in elucidating the development of carious attack has been its study at successive levels of intensity or extent of attack, as shown by figures illustrated graphically in Fig. 9.

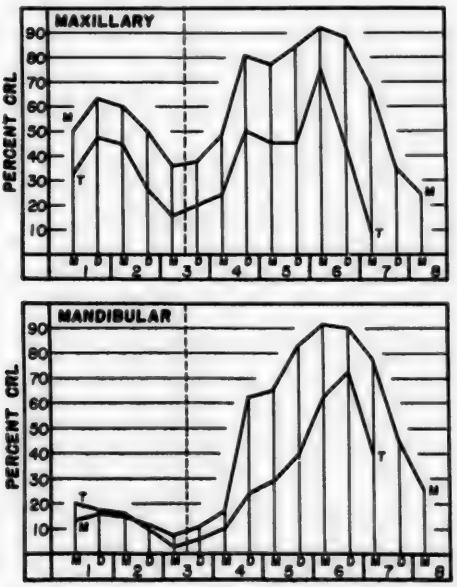


Fig. 7.—Development of proximal caries attack shown by study at different age levels. Profile graph showing caries experience in permanent dentitions of two age groups, both characterized by a high level of carious attack. From Table III. Series T (median age 13.5 years) and Series M (median age 20.0 years).

7. Relative Susceptibility of Proximal Surfaces

By means of exhaustive review of these and other unpublished component statistics for developing caries on proximal surfaces and by the superimposition of many profile graphs, it has been possible to consolidate many separate findings into the form of a general ranking of the separate proximal tooth surfaces of permanent teeth according to their relative susceptibility to caries. This ranking is given by regions (Table V) in view of the interregional relationships shown earlier. It is of interest to note that the combined findings for each of the surfaces of the permanent teeth (series TMA, all dentitions, Table III) conform well to the grouped ranks assigned to the various proximal surfaces (Fig. 10).

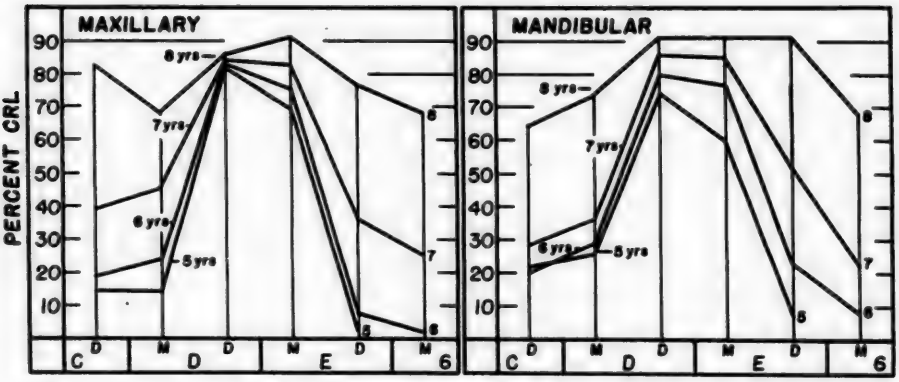


Fig. 8.—Progressive development of proximal caries attack shown by study at successive age levels. Profile graph showing caries experience in posterior teeth of mixed dentitions studied through successive age levels (5, 6, 7, and 8 years). From unpublished data, Series X, 122 cases.

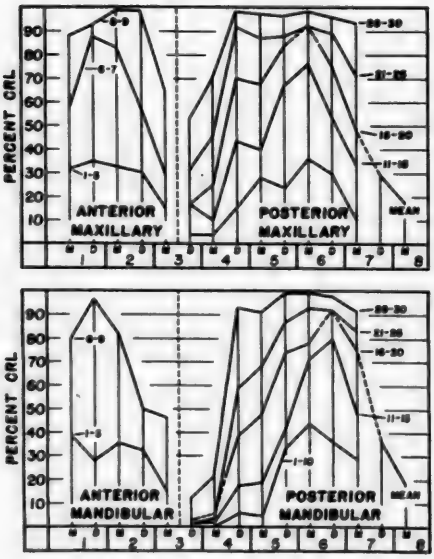


Fig. 9.

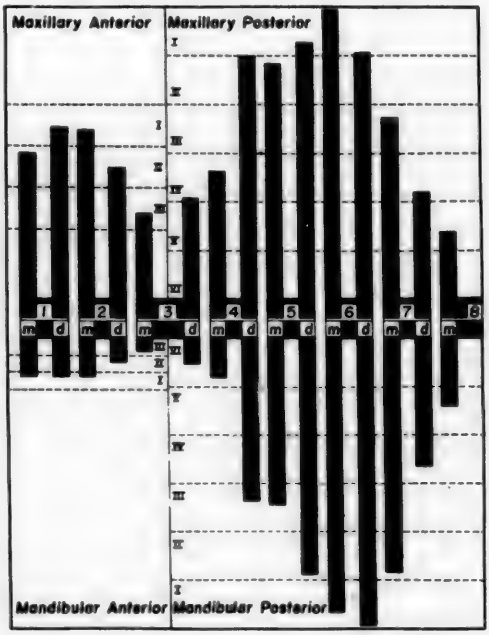


Fig. 10.

Fig. 9.—Progressive development of proximal caries attack shown by increments between different levels of total caries incidence. Profile graph showing caries experience in permanent dentitions, grouped according to total numbers of proximal lesions in the case of each region of the mouth. From unpublished data, Series M and A, 207 cases grouped as in Table III.

Fig. 10.—Relative caries susceptibility of the proximal surfaces. Bar graph compiled from data in Table III, Series TMA combined; with grouped ranks for caries susceptibility (from Table V) superimposed in each region (horizontal lines).

TABLE IV

CARIES EXPERIENCE (C. R. L.) OF INDIVIDUAL PROXIMAL SURFACES IN THE MIXED DENTITION
One Hundred Twenty-two Cases, Series X, 5 to 8 Years (Age Last Birthday)

	MAXILLA (%)	MANDIBLE (%)
C distal	31.5	27.0
D mesial	89.1	35.2
D distal	78.7	82.4
E mesial	34.4	78.2
E distal	24.2	37.7
6 mesial	12.7	17.6

TABLE V

PROXIMAL SURFACES OF THE PERMANENT TEETH RANKED BY GROUPS IN ORDER OF CARIES SUSCEPTIBILITY

(a) MAXILLARY ANTERIOR TEETH	(b) MANDIBULAR ANTERIOR TEETH
1. #1 distal #2 mesial	1. #1 mesial #1 distal #2 mesial
2. #1 mesial #2 distal	2. #2 distal
3. #3 mesial	3. #3 mesial

(c) POSTERIOR MAXILLARY AND MANDIBULAR TEETH	
1. #6 mesial (maxillary) #5 distal (maxillary) #6 distal (maxillary)	#6 distal (mandibular) #6 mesial (mandibular)
2. #4 distal (maxillary) #5 mesial (maxillary)	#5 distal (mandibular) #7 mesial (mandibular)
3. #7 mesial (maxillary)	#4 distal (mandibular) #5 mesial (mandibular)
4. #4 mesial (maxillary) #3 distal (maxillary) #7 distal* (maxillary)	#7 distal* (mandibular)
5. #8 mesial* (maxillary)	#8 mesial* (mandibular)
6.	#3 distal (mandibular) #4 mesial (mandibular)

*True susceptibility of these surfaces uncertain; as in many of the cases studied, they had only been in approximation with adjacent teeth for a relatively short posteruption period.

Attention is directed to the contrasting series M and A (Fig. 11). The caries profiles are essentially similar in form throughout, two notable differences being the caries incidence at a uniformly lower level in Series A by comparison with Series M, and the virtually complete absence of mandibular anterior proximal caries in Series A. These observations provide an interesting but scarcely helpful insight into the caries susceptibility and incidence of comparable North American and New Zealand populations—both Series A and Series M were composed of university students in those two countries respectively, and the median ages of these groups were comparable (see Table I). The suspected relatively high incidence of caries in New Zealand is clearly confirmed. There is no indication of an explanation beyond the fact that there is so much in common in the two patterns of carious attack that fundamental causes appear to have been similar in character, operating at different levels of intensity.

8. Proximal Restorations

The examination of these patients offered information on the presence of carious defects arising through failure of proximal restorations. The author has previously reported that in deciduous posterior teeth, simple "spot" proximal fillings had associated carious defects approximately twice as often

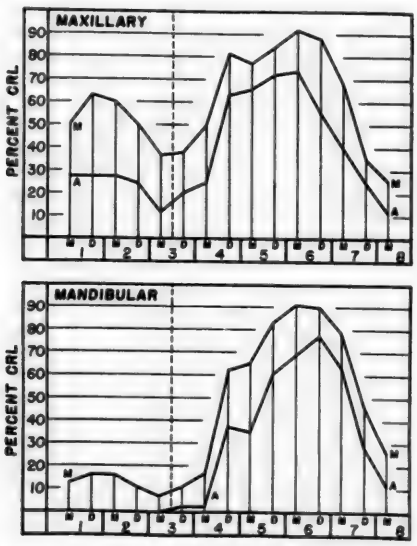


Fig. 11.—Patterns of caries attack on proximal surfaces in groups with contrasting levels of mean caries incidence. Profile graph showing parallel patterns for two groups of comparable age but characterized by different levels of mean caries incidence. From Table III, Series M and Series A.

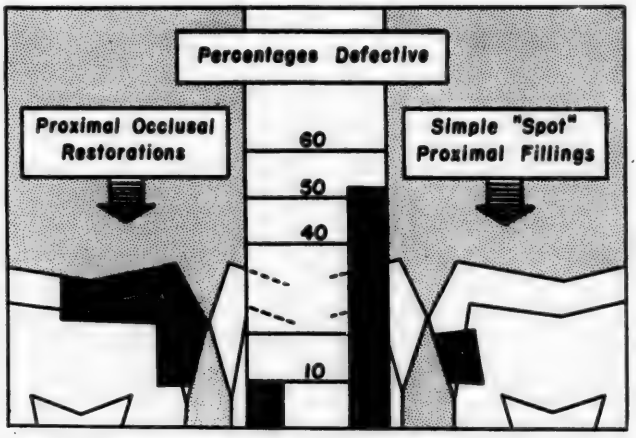


Fig. 12.—Effectiveness of occlusal-proximal two-surface restorations and of spot proximal fillings. (See text).

as conventional occlusal-proximal restorations. In the deciduous teeth, restorations are only present for a limited time and patients in the previously reported series were not above 8 years of age. To this finding may now be added another striking picture revealed in the case of permanent posterior teeth where many of the proximal restorations had been present for longer periods. In Series M, the short-cut spot filling procedure was shown to have been attended by carious failure five times as frequently (the exact figures were 52.2 and 10.2) as the conventional occlusal-proximal restoration (Fig. 12). In fairness to the possibilities with the use of gold foil, it must be stated that practically none of the spot fillings in the series studied were foil restorations.

CONCLUSION

Requirements of statistical significance do not permit of further analysis or interpretation of the data obtained from the series of cases studied. The findings invite confirmation or correction through a more extensive survey, especially one designed to embrace suitable continuous series of age groups. The present survey has indicated some of the characteristics of proximal carious attack and has served to suggest procedure for its further study.

SUMMARY

From a study of 20,816 proximal surfaces of teeth from mixed and permanent dentitions, of which 9,018 showed evidence of carious attack, it was found that:

1. The pattern of proximal caries attacks on individual teeth displayed a striking measure of bilateral symmetry for the aggregate experience of whole groups of cases, but that this bilateral symmetry of the total proximal caries pattern was seldom encountered in individual cases.

2. (a) There was high correlation between total maxillary and total mandibular proximal caries incidence in posterior teeth of individual cases. (b) In the presence of maxillary anterior proximal caries, there was a significant correlation between its total frequency and that for all posterior surfaces in individuals. Absence of maxillary anterior proximal caries was not, however, associated with any particular level of posterior proximal caries. (c) Presence or absence of mandibular anterior proximal caries showed no significant interregional correlations.

3. Approximation of surfaces was obviously a factor in proximal caries incidence, and a consistently high percentage of proximal attacks occurred as adjacent pairs of approximated attacks.

4. No clear picture emerged related to age or sex factors.

5. There are important difficulties to be overcome in selecting a basis for assessment of proximal surfaces as carious, under such circumstances as when teeth are missing or have been lost from the dentition.

6. By study of the total and incremental patterns of proximal carious attack, it was possible to rank the various proximal surfaces according to their relative caries susceptibility.

7. Essentially similar patterns of tooth susceptibility were characteristic in both slowly and rapidly developing carious attack on proximal surfaces.

8. Simple spot proximal fillings appeared to have become defective with five times the frequency of conventional occlusal-proximal two-surface restorations.

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THE CONDITION OF THE TEETH AND THE ATTACHMENT APPARATUS IN TUBERCULOSIS

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Brooklyn, N. Y.

MANY texts and much literature have been written on dentistry, but very little mention is made in them of tuberculosis. Darlington and Salman remarked, "While the literature on tuberculosis is probably more voluminous and complete than on any other single condition or disease, that, which we have found on tuberculosis in relationship to dentistry, is, in comparison, meager and less scientific."[1]

Tuberculosis is one of the most widespread diseases afflicting humanity today. In the year 1945, there were over 117,000 new cases of tuberculosis reported and almost 53,000 deaths in the United States directly attributable to tuberculosis.[2] Tuberculosis may affect almost any organ of the human body. It must, therefore, be of interest to any profession dealing with human ailments.

Most dentists have never thought of tuberculosis in terms of dentistry. Today, however, the dentist plays a vital role in treating the tuberculous patient. Generally speaking, the treatment for tuberculosis is diet, rest, and sunshine. It is impossible for a beneficial diet to be administered if the patient is lacking in sound and sufficient masticators. No treatment that the sanatorium can offer is effectual if nutrition and rest are impossible, and nutrition and rest are impossible in even a decent degree for a patient with a foul mouth or one suffering from toothache.

In reviewing the literature on tuberculosis, it was found that opinions regarding the possible changes in the dental apparatus were rather divergent; some investigators believing there is a characteristic oral disturbance[3-11] and others holding there is no relationship whatsoever.[12-17] The problem seems to resolve itself into this question: are there any oral signs which could be established as pathognomonic of tuberculosis? Those authors who believe that there is a direct relationship between the condition of the teeth and attachment apparatus and tuberculous infection state that there is a cervical type of cavity and some form of gingivitis characteristic of tuberculosis. In this cervical cavity or, as it has sometimes been called, the "circular" cavity or the erosion cavity, the dentin is soft, leathery, and of a light brownish color. Pain may or may not be associated with caries.

Most of the aforementioned investigators came to their conclusions without the aid of a clinical study, at least no such study is mentioned in their reports.

A preliminary report was presented in part at the Twentieth General Meeting of the International Association for Dental Research, New York City, March 14 and 15, 1942.

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ORIGINAL STUDIES*

Selection of Cases

The patients had pulmonary tuberculosis varying in duration from one month to eight years. The majority of the patients had the diagnosis made within the past year. All stages of the disease were represented.

Two hundred patients were examined (117 males and 83 females) ranging in age from 16 to 66. The patients were of the middle and lower third classes. There were 141 white patients (84 male, 57 female), 51 Negroes (28 male, 23 female), 7 yellow (5 male, 2 female), and one female American Indian.

Method

A special chart was devised and the clinical data were recorded by means of a code.

Plain mirrors (No. 5) and Nos. 7 and 17 explorers were used. The lips, tongue, palate, and the buccal mucosa were examined first. The teeth present in the mouth were then examined along with the corresponding gingivae. Unerupted and extracted teeth were noted, as well as any prosthetic appliances. A tooth was considered carious when there were necrosis and debris associated with cavitation on that surface of the tooth which is exposed in the oral cavity. Defects which did not answer the requirements of this definition were not recorded as dental caries.[18] Pits and fissures in which the explorer caught, but which were not considered to be carious, were not counted as caries. The amount of calculus and the position it occupied on the tooth were then charted. The state of the oral hygiene was recorded as good, fair, or poor. At the completion of the examination, the amount of saliva was recorded. The amount was estimated by the quantity that had been present during the examination.

Age and Sex Distribution

Patients were divided into four age groups: 16 to 20, 21 to 30, 31 to 40, and 41 and higher. There were 35 in the 16 to 20 age group (15 male, 20 female), 77 between 21 and 30 years of age (38 male, 39 female), 52 in the 31 to 40 age group (36 male, 16 female), and 36 over 40 years of age (28 male, 8 female).

Before proceeding with the analysis of the data, it is deemed essential to mention the character of dental treatment the patients received at these hospitals. A dentist was in attendance one morning a week at the Brooklyn Thoracic Hospital. At Sea View Hospital, two interns and two residents were in daily attendance and there was also a large visiting staff. The treatment administered was palliative and conservative, that is, extractions were not the rule. A tooth was extracted only if it was definitely indicated from a dental viewpoint.

The results which follow are defects per individual. These were obtained by computing the average. Due to the large amount of data, only figures which are considered significant will be discussed.

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*The original studies were made at the Brooklyn Thoracic Hospital and Sea View Hospital, Staten Island, N. Y.

DATA

Oral Hygiene

The oral hygiene encountered was fair in 42 per cent, good in 30 per cent, and poor in 28 per cent of the cases. It was better in females than in males. As age advanced, the oral hygiene became poorer.

Salivary Calculus

Calculus was absent in 25.5 per cent of the cases studied; 35.5 per cent had a slight amount; 23 per cent, a moderate amount; and 16 per cent, an excess amount. In the 16 to 20 age group, calculus was absent in most of the cases; in the 21 to 30 age group, a slight amount predominates; and in the 31 to 40 age group, a moderate amount. Where calculus was found, it was subgingival in 27 per cent of the patients, at the gingivae in 71 per cent, and supragingival in 2 per cent.

The state of oral hygiene and the amount of calculus that were found appear to be closely correlated. This can be seen from the data in Table I.

TABLE I

COMPARISON OF SALIVARY CALCULUS AND STATE OF ORAL HYGIENE

CALCULUS		ORAL HYGIENE	
AMOUNT	% OF TOTAL	STATE	% OF TOTAL
Absent	25.50	Good	30.15
Slight	35.50	Fair	42.21
Moderate plus Excess	39.0	Poor	27.63

The oral hygiene was in a better state in females than in males. Likewise, less salivary calculus was found in females. As age increased, more calculus was found and the oral hygiene became poorer.

Saliva

The amount of saliva was slight in 54 per cent of the cases; moderate in 37 per cent; in excess, in 9 per cent of the cases. The observed amount of salivary flow increased with an increase in age, and was greater in males than in females.

Sound, Carious, and Missing Teeth

Figures for sound, carious, and missing teeth were determined on the basis of a full complement of 32 teeth.

Sound teeth.—Teeth designated as being sound were those devoid of caries, fillings, root canal fillings, or any other hard structure defect, as well as any periodontal disease.

More than two-fifths of the full complement of teeth (43 per cent per individual) were considered to be sound. Females had approximately 15 per cent more sound teeth than males. This was equally true in each age group studied, except the 21 to 30 age group in which the amount was about the same. There was a continuous decrease in the number of sound teeth as age increased. These data are recorded in graphic form on Fig. 1. The detailed tabulation of these data is given in Table II.

TABLE II
THE NUMBER OF SOUND TEETH IN RELATION TO AGE AND SEX

AGE GROUP	MALE		FEMALE		AVERAGE	
	NO. PER CASE	% PER CASE	NO. PER CASE	% PER CASE	NO. PER CASE	% PER CASE
16 to 20	16.33	51.03	20.25	63.28	18.57	58.03
21 to 30	16.05	50.16	16.62	51.94	16.34	51.06
31 to 40	10.22	31.94	14.38	44.94	11.50	35.94
41 and higher	4.89	15.28	10.13	31.66	6.06	18.94
Totals	11.62	36.31	16.43	51.34	13.64	42.63

Missing teeth.—Included under missing teeth were unerupted, congenitally missing teeth, and those which had been extracted. Approximately one-fourth of the teeth, 7.83 (24 per cent per individual), were found missing. A 9 per cent higher incidence was found in males than females, 28 per cent in males and 19 per cent in females. Males have a higher incidence of missing teeth in all age groups except the last. As age increases, there is a corresponding increase in the number of missing teeth. The high per cent, 14, in the 16 to 20 age group, is attributable to the fact that unerupted teeth were considered as missing teeth.

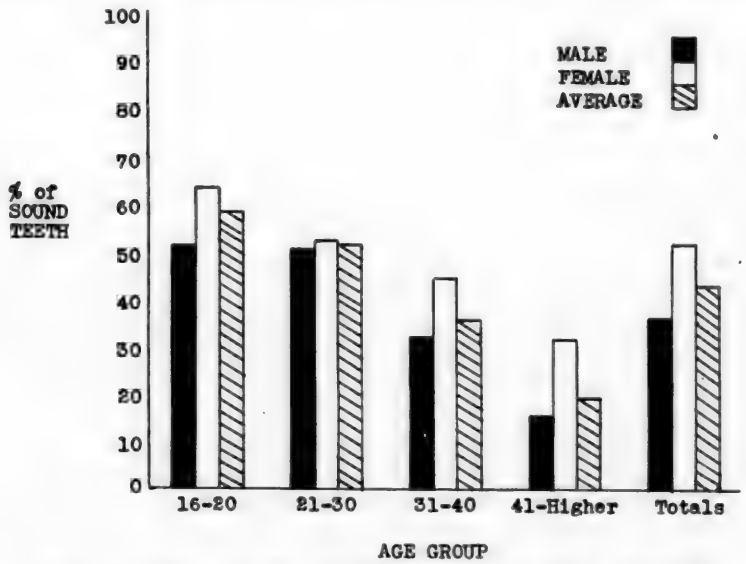

Fig. 1.—Incidence of sound teeth in different age groups (200 cases).

Carious teeth.—Teeth which had fillings, caries, or crowns were grouped under carious teeth. In the total cases examined, 24 per cent of the teeth per individual were considered carious. There is a higher incidence of carious teeth in males in the 16 to 20 and 41 and higher age groups; in the 31 to 40 age group, the incidence is higher in females. Of the total cases examined, the incidence of carious teeth is approximately 1 per cent higher in females than in

males. As age increases, the number of carious teeth remains approximately the same. There is, however, a slight fluctuation. Loss of teeth due to caries occurs more frequently in the older groups, so that the number of carious teeth in all the age groups remains about the same. The detailed tabulation of these data is given in Table III.

TABLE III

THE NUMBER OF CARIOUS TEETH IN RELATION TO AGE AND SEX

	MALE		FEMALE		AVERAGE	
AGE GROUP	NO. PER CASE	% PER CASE	NO. PER CASE	% PER CASE	NO. PER CASE	% PER CASE
16 to 20	8.27	25.84	7.10	22.19	7.89	24.66
21 to 30	8.63	25.97	8.92	27.88	8.78	27.44
31 to 40	7.31	22.84	9.06	28.31	7.85	24.53
41 and higher	5.86	18.31	3.38	10.56	5.31	16.59
Totals	7.60	23.75	7.98	24.94	7.76	24.25

Number and type of cavities.—The number of cavities was found frequently to be greater than the number of carious teeth since a tooth may have more than one cavity. Both virgin cavities and those that had been filled were included in this category. The general rule for classification of cavities was followed with these exceptions: (1) a mesio-occluso-distal cavity was designated as two Class II cavities; (2) a bucco-occluso-lingual cavity as two Class I cavities; (3) a disto-occluso-lingual, a disto-occluso-buccal, a mesio-occluso-buccal, or a mesio-occluso-lingual cavity as one Class I and one Class II cavity, and (4) a remaining shell, as two Class I cavities and two Class II cavities.

There were 10.86 cavities found per individual. There is a higher incidence of 0.27 cavities per individual in females than in males. In the 16 to 20 and 41 and higher age groups, there is a higher incidence in males. As age increases, the number of cavities remains about the same. The detailed tabulation of these data is given in Table IV.

TABLE IV

THE NUMBER OF CAVITIES IN RELATION TO AGE AND SEX

	MALES	FEMALES	AVERAGE
AGE GROUP	NO. PER CASE	NO. PER CASE	NO. PER CASE
16 to 20	12.20	9.10	10.43
21 to 30	11.32	12.90	12.22
31 to 40	10.06	11.63	10.54
41 and higher	9.79	5.38	8.81
Totals	10.74	11.01	10.86

The incidence of the number of Class V cavities is very small as compared to the total number of cavities.

Fig. 2 illustrates that there is a greater incidence of the Class I cavity than any of the others. There were 4.64 Class I cavities per individual; 3.14 Class II cavities; 1.51 Class III's; 0.23 Class IV's and 1.36 Class V cavities. The supposedly characteristic Class V or cervical cavity was fourth in frequency, only 1.36 per individual. Four of the patients are chiefly responsible for this high

result of Class V cavities. These four patients had approximately one-fifth of the total Class V cavities found. Only four of the two hundred patients examined showed a high incidence of Class V cavities.

Class I and II cavities predominate in females, and the Class III and V cavities in males. The Class IV cavity is about the same in either sex (Fig. 2). The number of Class I cavities increases very slightly at first with age; but then with the further increase of age, it begins to decrease. The other types of cavities increase with age, except for slight fluctuations. The decrease in the number of Class I cavities as age increases, with the subsequent increase in Class II's, may be explained on the basis that the Class I becomes a Class II cavity. Therefore, in the older groups, there are less Class I's and more Class II cavities.

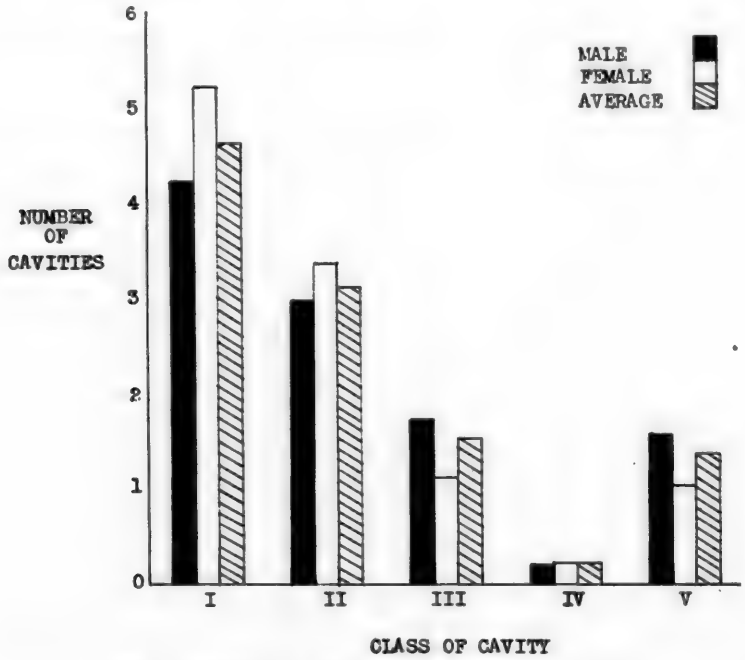

Fig. 2.—Incidence of the different cavities (200 cases).

D.M.F. surfaces involved.—The term "D.M.F. surfaces involved" is introduced as a simple and convenient method of summarizing the tooth structure changes. It was originally introduced by Klein, Palmer, and Knutson[19] to designate the complete caries experience. The "D" signifies decayed; the "M" signifies missing; the "F," filled. For each surface of the tooth involved there is one D.M.F. surface involved. Residual roots and full crowns are each counted as five D.M.F. surfaces involved, and missing teeth as three D.M.F. surfaces involved.

In the total cases examined, 38.73 D.M.F. surfaces were involved per individual. The D.M.F. was approximately 9 higher in males than in females.

The higher result in males was likewise found in every age group studied. With an increase in age, there was a corresponding increase in the D.M.F. value obtained, both in males and females. This is recorded in graphic form on Fig. 3. The detailed tabulation of these data is given in Table V.

TABLE V

THE NUMBER OF D.M.F. SURFACES INVOLVED IN RELATION TO AGE AND SEX

AGE GROUP	MALE NO. PER CASE	FEMALE NO. PER CASE	AVERAGE NO. PER CASE
16 to 20	29.80	24.70	26.89
21 to 30	34.11	33.46	33.78
31 to 40	44.33	34.44	41.29
41 and higher	58.61	52.0	57.14
Totals	42.56	33.33	38.73

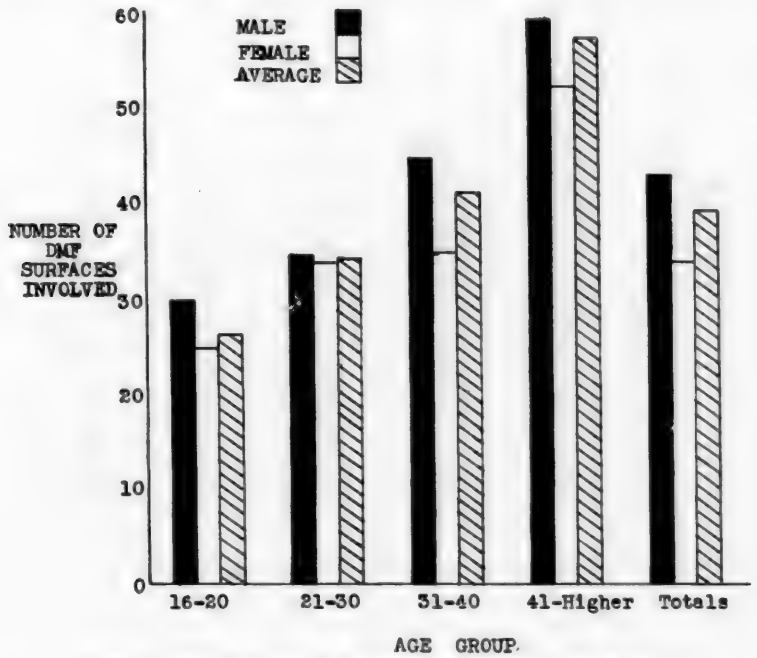

Fig. 3.—D.M.F. incidence in different age groups (200 cases).

Hypoplasia of the Enamel

Hypoplasia was found in 13 of the 200 cases examined. Only 39, or 0.6 per cent of the total expected teeth, were considered hypoplastic. The middle third of the crown was most often affected. Occasionally more than one-third of the crown was involved.

Soft Structures

To simplify study of the periodontal tissues, this was divided into: (1) healthy-appearing periodontal tissue, and (2) its position at the amelo-cemento junction.

Healthy appearing periodontal tissue.—To explain what is meant by healthy-appearing periodontal tissue, it will probably be easier to describe what was considered unhealthy tissue. Any tissue was recorded unhealthy if it showed any of the following: any form of gingivitis, edema, hyperemia, hypertrophy, exudation, or ulceration. Recession of the gingival tissue was not considered in this category.

The periodontal tissues were considered healthy in 169 (85 per cent) of the cases examined. The incidence of healthy-appearing tissue was greater in females than in males. Both sexes, however, possessed high per cents of healthy tissue. The results obtained in the various age groups studied indicate very healthy tissue, irrespective of the age, though there is a slight decrease with increasing age (Fig. 4).

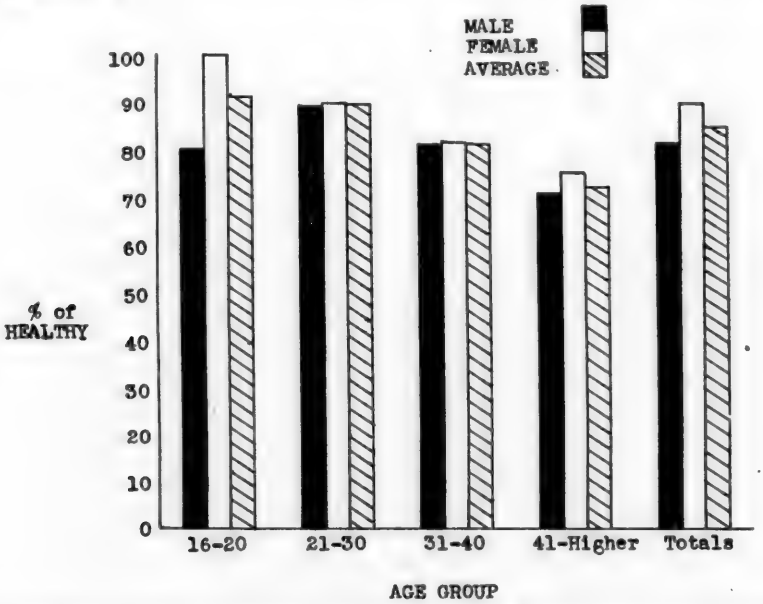

Fig. 4.—Incidence of healthy appearing periodontal tissue in different age groups (200 cases).

Position of the periodontal tissue at the amelo-cemento junction.—The position of the periodontal tissue at the amelo-cemento junction was considered either normal (no recession), or else presenting slight, moderate, or marked recession. Generalized recession was spoken of as recession around all the teeth present, localized, as being around one or more teeth, but not all of them.

The position of the periodontal tissue was normal (no recession) in 68 per cent of the cases studied. Many of the cases charted as abnormal showed recession only around one or two teeth. In most cases where any recession was seen, it usually was slight. Only 19 per cent of the cases could definitely be classified as generalized recession, 14 per cent as localized. All types of recession were more prevalent in males. The incidence of the various types of recession in relation to sex is given in Fig. 5. As age increased, recession became more common.

Alveolar Destruction

The only clinical method of studying the alveolar process is through the agency of the x-ray. However, it was impossible to obtain roentgenograms of these patients and, therefore, another criteria had to be established. Rather than call it "alveolar absorption" the term "alveolar destruction" was substituted. Loose teeth were considered indicative of alveolar destruction. The amount of destruction was determined by the amount of mobility.

Only 135, or slightly more than 2 per cent of the teeth examined were considered mobile. A few patients accounted for most of the mobile teeth.

Oral Lesions

There were no cases which presented themselves with oral tuberculous lesions.

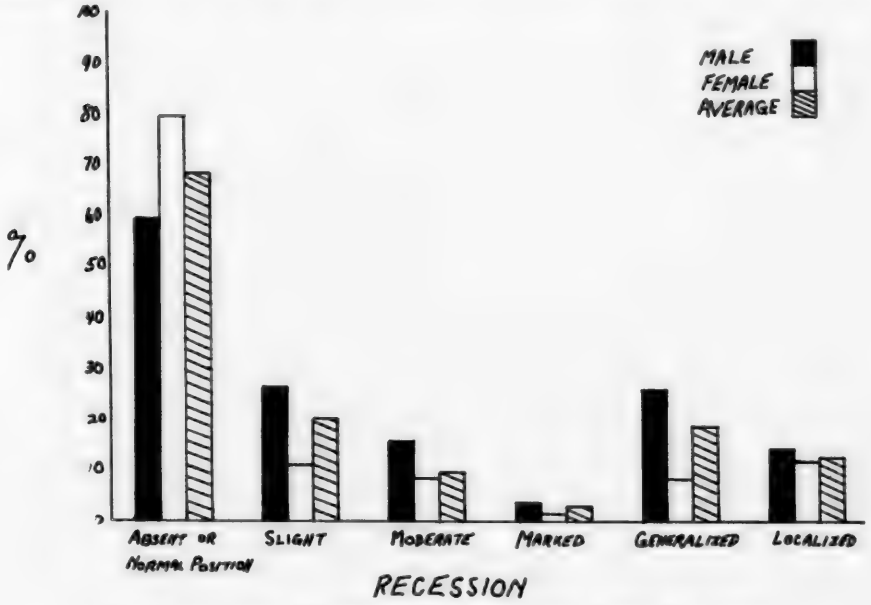

Fig. 5.—Incidence of recession of the periodontal tissue (200 cases).

DISCUSSION

There was noted a higher incidence of carious teeth and of cavities in females than in males. This is generally expected among nontuberculous people.[20, 21] Bobtscheff[8] in his study of tuberculous individuals found results very similar to those obtained in this investigation. He found approximately 5 per cent less missing and carious teeth than were found in this investigation. Yet, he believed that caries is more prevalent in the tuberculous than in the nontuberculous individual.

Only four of the 200 cases examined showed a high incidence of the Class V cavity. This is the cavity which is held to be characteristic of tuberculous patients. From the results obtained this cavity is not characteristic of tuberculous patients.

The D.M.F. values of supposedly healthy individuals* are very similar to those obtained in this investigation. Though the age groups are not similar, they are close enough to warrant comparison. Table VI indicates that the D.M.F. is higher in tuberculous patients than in healthy persons in only two of the four age groups studied. In the 16 to 20 age group, the results of this investigation are slightly higher than those obtained by Klein, Palmer, and Knutson.[19] In the 21 to 30 age group, the difference is so small that it is probably of no significant value. In the two older age groups, Klein, Palmer, and Knutson's D.M.F. was higher than was found in tuberculous patients. Klein, Palmer, and Knutson found an increase in the D.M.F. as age increased. This was likewise the case in tuberculous patients. It is apparent that there is no marked variation of the D.M.F. in tuberculous patients and in supposedly healthy individuals. Therefore, we must assume that there are no hard structure defects characteristic of tuberculosis.

TABLE VI

THE NUMBER OF D.M.F. SURFACES INVOLVED IN HEALTHY AND IN TUBERCULOUS INDIVIDUALS IN RELATION TO AGE

KLEIN, PALMER, AND KNUTSON		GRUBER	
AGE GROUP	NO. PER CASE	AGE GROUP	NO. PER CASE
17 to 19	23.48	16 to 20	26.89
20 to 29	33.54	21 to 30	33.78
30 to 39	49.33	31 to 40	41.29
40 to 59	58.91	41 and higher	57.14

Approximately 85 per cent of the individuals examined had healthy appearing periodontal tissue. This value appears to be quite high, perhaps higher than one might expect to find in healthy individuals. Cipes[4] was of the opinion that there is more periodontal disease in the tuberculous than in the nontuberculous person. He came to this conclusion after studying over one thousand cases. He found 19 per cent had normal gingival tissues and 74 per cent had recession, gingivitis, or true periodontosis. The results obtained in this investigation obviously do not coincide with those of Cipes. Of the cases Tanchester and Sorrin[14] examined, 46 per cent had some form of gingivitis. Yet their conclusion was that there is no more periodontal disease in tuberculous than in nontuberculous cases. That the incidence of healthy appearing periodontal tissue is greater in females than in males coincides with the results obtained by Miller and Seidler[21] on healthy individuals. From this investigation, it appears that periodontal disease is not necessarily found more often in tuberculous than in nontuberculous individuals.

Perhaps the cases of generalized and localized recession that were observed were caused by the disease; but can they be considered as being pathognomonic of tuberculosis? Thirty-seven cases of generalized recession and twenty-seven of localized recession do not seem common enough to draw a conclusion that periodontal diseases are pathognomonic of tuberculosis. The incidence encountered does not seem higher than one would expect to find in healthy persons.

*Personal communication from Dr. H. Klein. (This D.M.F. value was computed by Klein and Palmer on 12,000 employees of the Metropolitan Life Insurance Company.)

Besides, these patients may have had recession long before the diagnosis of tuberculosis was made. It was impossible to determine the past dental history of most of these cases. One patient stated that he had just begun to notice the condition of his gums, that is, since admission to the hospital. When this patient was admitted, a brief dental history was taken by a physician. It was noted at that time, that his teeth were in dire need of repair and that his oral hygiene was very poor. Tuberculosis cannot be justly accused as the cause of the recession here, though it may have been a contributing factor. Other factors may have also played an important role in producing recession.

If there is any change in the alveolar bone in the tuberculous patient, it is not noticeable in the results obtained in this investigation. Cahn[3] reported that radiographically the intertrabecular spaces seem to be enlarged and swollen, as if the bony structure were being replaced by soft tissue, or, better yet, as if the phosphorous and lime salts of the bone were disappearing, leaving only the matrix.

It is not surprising that no oral tuberculous lesions were encountered. They are very rare and, if seen at all, are seen mostly in far-advanced cases of tuberculosis. Rubin[22] stated that the incidence of oral involvement in tuberculosis is 1.44 per cent. Only 5 cases of involvement of the gums and cheeks were observed by Martin and Koepf[11] in 3,835 admissions over a ten-year period. According to reports occurring in medical literature, lingual tuberculosis is infrequent, only 357 cases being recorded up to January, 1936.[23] In the mouth, the tongue is the favorite seat of tuberculous ulcerations. Males are more frequently affected than females, the incidence varying from 3 to 2, to 23 to 1.[24] Apparently the mouth does not afford the conditions necessary to the growth of the bacillus with the subsequent development of oral lesions. Saliva may play an inhibitory effect,* and there evidently must first be a break in the epithelium.[25]

SUMMARY AND CONCLUSIONS

A study of the hard and soft tissue changes in 200 adults has been reported. The findings were subjected to statistical evaluation. Related data reported in the literature have been correlated whenever possible.

The Class V cavity does not appear to be characteristic of tuberculous patients. The D.M.F. value obtained indicated no higher incidence of hard structure defects in tuberculous patients as compared to that obtained in supposedly healthy individuals. The high incidence of healthy appearing periodontal tissue and the slight amount of recession encountered do not seem to indicate any unusual periodontal involvement in the tuberculous patient. The D.M.F. was found higher and there was more periodontal involvement in males than in females. As age increased, there was a corresponding increase in the D.M.F. as well as in the amount of periodontal involvement.

Inasmuch as so many variables enter into an investigation of this type and inasmuch as so few cases were examined, no conclusions are warranted. However, it is apparent that there are no oral signs which could be established as pathognomonic of tuberculosis.

*Personal communication from Mrs. A. K. Dietz and Dr. F. Kantor.

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CARIOUS LESIONS IN COTTON RAT MOLARS

V. An Effect of the Type of Cage and Bedding on the Initiation and Development of Carious Lesions

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THE cotton rats which were found to be susceptible to tooth decay in the investigations of Shaw, Schweigert, McIntire, Elvehjem, and Phillips,[1] and Shaw, Schweigert, Elvehjem, and Phillips[2] had been maintained throughout the experimental periods in small individual cages with floors made of coarse wire screen. For the last four years, all experiments to study the relation of diet to tooth decay in cotton rats in this laboratory have been conducted in the above type of cage. Prior to weaning at 14 days of age for experimental purposes, the cotton rats in the investigations of Shaw and his co-workers[1, 2] and in this laboratory had been allowed to run with their parents in the breeding cages where the sheet metal floors were covered with wood shavings. During the process of evaluating the number and extent of carious lesions in supposedly highly caries-susceptible cotton rats at the end of a variety of experiments, certain animals were found to have an unexpectedly higher resistance to the initiation and development of carious lesions than their littermates. The cotton rats with high resistance to tooth decay commonly had appreciable amounts of fibrous debris in the sulci of their first and second molars. The sulci of the third molars rarely contained such material except in the case of cotton rats which were maintained in stock colony cages until 5 or 6 weeks of age, prior to which time the third molars either had not erupted or were not in occlusion. The debris in the sulci was composed of what grossly and histologically appeared to be fragments of wood shavings mingled with considerable amounts of short hair. Since wood shavings could only have been obtained prior to removal of the cotton rats from the stock colony cages, the particles must have been retained in the narrow sulci throughout the experimental period. In all probability, the hair had been ingested along with the shavings or in the process of cleaning the hair coat. The older the cotton rats were before removal from the breeding cages for experimental purposes, the more frequently, and more extensively, this impaction of foreign material into the sulci appeared to occur. These observations suggested that an experiment be conducted where weanling cotton rats were maintained on a caries-producing dietary regimen under a variety of caging circumstances for the usual period of 14 weeks.

EXPERIMENTAL

The cotton rats used in these experiments were raised in our stock colony, and weaned at about 14 days of age.[3] In one experiment, the litters were

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divided into two groups. The cotton rats in the first group were placed in individual cages with screen floors, while those in the second group were distributed in twos and threes of one sex in large cages, the sheet metal bottoms of which were covered with relatively coarse wood shavings. In a second experiment, a series of litters was divided into two groups. The cotton rats in the first group were maintained in the small, individual cages with screen bottoms, while those in the second group were distributed into larger cages where the sheet metal bottoms were covered with long, narrow strips of newspaper (paper clippings) as the sole bedding. All four groups of cotton rats were offered Ration 100 and water ad libitum. This ration was composed of 67 per cent sucrose, 24 per cent purified casein, 5 per cent corn oil, and 4 per cent salt mixture, supplemented with adequate amounts of thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, nicotinic acid, choline chloride, inositol, para-aminobenzoic acid, beta-carotene, irradiated ergosterol, 2-methyl-1, 4-naphthoquinone, alpha-tocopherol, and liver concentrates to permit normal growth.[3]

At the end of 14 weeks on experiment, the cotton rats were sacrificed, the heads fixed in 95 per cent ethanol, and evaluated for number and extent of carious lesions according to previously established criteria.[1, 2]

TABLE I

THE AVERAGE NUMBER AND EXTENT OF CARIOUS LESIONS OBSERVED IN COTTON RATS MAINTAINED FOR FOURTEEN WEEKS IN CAGES WITH DIFFERENT TYPES OF FLOORS AND BEDDING

TYPE OF BEDDING	NUMBER OF COTTON RATS	AVERAGE NUMBER OF CARIOUS LESIONS	S.E.M.*	C.R.†	AVERAGE EXTENT OF CARIOUS LESIONS	S.E.M.*	C.R.†
None	7	33.7	1.3	6.0	110+	6+	6.1
Wood shavings	10	17.8	2.3		39+	8+	
None	9	27.2	2.8	0.9	80+	12+	0.9
Paper clippings	10	24.1	2.0		67+	8+	

*Standard error of mean.
†Critical ratio.

RESULTS

The average number and average extent of carious lesions observed in each of four groups of cotton rats are presented in Table I. The cotton rats, which had been maintained in large cages with wood shavings as bedding, had a significantly lower average number and a significantly lower average extent of carious lesions than their littermates which were maintained in cages with floors of coarse wire screen. There had been a 50 per cent reduction in the average number of carious lesions, and a 65 per cent reduction in the average extent of carious lesions in the cotton rats maintained with wood shavings as bedding. Thus, not only the average number and average extent of lesions were reduced, but each lesion had developed to a lesser degree than in littermates in cages with floors of coarse wire screen. However, there was no significant difference between the average number and average extent of carious lesions in the cotton rats maintained on screen floors, versus those maintained in cages with newspaper clippings for bedding.

Examination of the molar teeth during the process of evaluating carious lesions indicated that there had been appreciable accumulations of closely packed debris in the sulci of those cotton rats allowed to run in bedding of wood shavings throughout the experiment. The debris was composed of small fragments of wood shavings closely packed together and intermingled with short hairs. The amount varied greatly from animal to animal but was relatively constant for all molar teeth in any cotton rat. These accumulations of wood shavings were not accompanied by fracture of the cusps. The particles were apparently small enough to have been pressed into the sulci without detectable damage. A few of the sulci of the molars in those cotton rats maintained in cases with paper clippings contained some materials of a different and rather soft, pulpy nature. In addition to the infrequency of these accumulations, there was neither as dense packing nor as much material. Fewer hairs were found to be intermingled with this type of debris. The sulci of the molars in both groups of cotton rats maintained in cages with screen floors were almost completely free of any materials other than residues of the ration with occasional clumps of hair.

The average rates of growth, and the final body weights, for the four groups of cotton rats in this investigation were optimal and did not differ from one another by any significant amount. Appearance, behavior, and activity were normal for all groups.

DISCUSSION

An experiment of this type clearly emphasizes the exactness with which an experimental regimen must be controlled in investigations on the production of tooth decay in laboratory animals. It is impossible from the present study to determine the mechanism whereby a reduction in the initiation and development of carious lesions was produced in caries-susceptible cotton rats maintained in cages with wood shavings as bedding. On the basis of these data, the appreciable accumulations of wood shavings and hairs in the sulci of the molars should not necessarily be interpreted to indicate that the presence and location of the debris was the direct causative factor of the lower dental caries experience. Since the accessibility of feces and urine for ingestion was equal for the animals maintained on bedding of wood shavings or of paper clippings, apparently the consumption of these materials was not of importance in the production of the reduced initiation and development of carious lesions among the cotton rats in the former group. Since the rates of growth and the final body weights were similar for all groups, a restriction in food intake by the cotton rats maintained in cages with wood shavings as bedding could not be implicated as a causative factor of the reduced amount of tooth decay.[4]

SUMMARY

The initiation and development of carious lesions in cotton rats was influenced by the type of cage and bedding in which they were housed during the experimental period. Cotton rats maintained in cages with screen floors

had a significantly higher average number and a significantly higher average extent of carious lesions than littermates maintained throughout the experiment in cages with sheet metal floors covered with coarse wood shavings. Cotton rats, which were housed in cages with sheet metal floors covered with paper clippings, did not have an appreciably different dental caries experience from their littermates in cages with screen floors.

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BIOCHEMICAL PRODUCTION OF OXALIC ACID FROM CARBOHYDRATES

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THIS paper reports the production of oxalic acid from starch through the intermediary of salivary enzymes.

Oxalic acid has long been of interest to nutritionists and the dental profession because in the presence of available calcium it forms calcium oxalates. Kohman, in considering oxalic acid in foods, reported on a study in animals involving subcutaneous injection of oxalic acid.[1] He concluded that the calcium that combines with oxalic acid introduced into the blood is rather promptly replaced by calcium being mobilized from other tissues of the body and the toxic symptoms are thus relieved. Ershoff stated that there seems to be no doubt that oxalates do interfere with efficient calcium utilization.[2] The relatively insolubility of calcium oxalate has long been of concern to the medical profession because of the precipitation of calcium oxalate in the renal tubules, ureters, and urinary bladder.[3]

As early as the writings of Lavoisier[4] starch was known as a source of oxalic acid. Madame Gruzewska[5] in 1912 listed oxalic acid as among the in vitro products of the oxidation of starch by H_2O_2. Loeper[6] discussed oxalic acid as an intermediate in carbohydrate breakdown. More recently, Caldwell and Hixon[7] have shown glyoxal $\begin{matrix}\text{CHO}\\|\\\text{CHO}\end{matrix}$ (one oxidative step from oxalic acid) to be an end product of acid hydrolysis of periodate starch. Girard, as reported by Loeper,[6] found that the oxidation of sugar is in three stages, namely—glycolic aldehyde, glyoxal, and oxalic acid. It has long been common manufacturing knowledge that cellulose is a source for the production of oxalic acid.[8] In spite of these observations, physiologically and biochemically, starch has had scant attention as a precursor of oxalic acid.

In this paper we report the production and measurement of oxalic acid from starch through the action of human salivary enzymes and through the action of alpha amylase (Wallenstein). Earlier findings[9] indicated that the saliva of individuals with extensive dental caries incubated with starch produces several times as much acid (as measured with .01 N NaOH) as the saliva of caries-free individuals. This finding stimulated the attempt to determine whether oxalic acid is among the acids produced from starch.

The analytical procedure was as follows: The saliva of twelve individuals with extensive active caries (8 to 10 cavities in the previous six months) was pooled. Fifty milliliters of this saliva was added to 50 ml. of 1 per cent corn starch. To this, 1 Gm. of calcium acetate powder was added.[10]

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The mixture was incubated at 37.5° C. for fifteen minutes. When cooled to room temperature 20 ml. of calcium acetate reagent made according to Bau[11] was added. The solution was placed in the refrigerator at 7° C. for forty-eight hours. At this time it was removed from the refrigerator and centrifuged; the fine white precipitate was washed thoroughly with distilled water with interim centrifuging. (Calcium lactate, calcium malate, calcium thiocyanate, calcium succinate, calcium acetate, calcium benzoate, calcium chloride, if present, may be expected in the wash water. Because of the relative insolubility of calcium oxalate, the latter remains as a precipitate.) After thorough washing with water, the precipitate was washed three times with NH_4OH (1:49) with interim centrifuging. After decanting the final NH_4OH wash, the tube was inverted for draining. The precipitate was then dissolved in H_2SO_4 (1:4) and heated to 80° Centigrade. Titration for oxalic acid was with .01 N $KMnO_4$ (standardized against .02 N NaOH) at a temperature between 60° C. and 80° C.[12, 13]

According to this measure, the action on starch of the saliva of these individuals with severe dental caries yielded 3.52 mg. of oxalic acid per Gm. of starch. This figure represents the difference between the titration of the saliva of these same individuals subjected to the same treatment and their salivary-starch production of oxalic acid. The figure was verified by several confirmatory runs (saliva from the same individuals at intervals of several weeks) yielding results which varied less than 10 per cent.

The above experimental procedure was repeated using alpha amylase as the digestive agent for starch. Digestion was carried out in an atmosphere of CO_2. The proportion of alpha amylase and starch was 1:1 by weight. The average production of oxalic acid in this case was 2.98 mg. of oxalic acid per Gm. of starch.

Controls of known oxalic acid were carried through with the foregoing tests. With this procedure we were able to recover only approximately 75 per cent of the starting amount of oxalic acid; therefore we must assume that the method employed did not indicate as much oxalic acid as was actually produced.

It is recognized that crystal forms are few and crystalline substances many, but repeated examination of crystalline material from saliva-starch and from amylase-starch has shown the presence of birefringent, monoclinic crystals which on the petrographic microscope conform to the appearance of crystals of known oxalic acid when crystallized under the same conditions of solvents and temperature.

The 3.52 mg. of oxalic acid per Gm. of starch from saliva-starch might at first appear to be very little but these individuals averaged a carbohydrate intake per day of 240 Gm. which yields a potential oxalic acid of 845 mg. daily. Since 1 mg. of oxalic acid is capable of taking 0.45 mg. of calcium out of solution, it is obvious that an appreciable amount of calcium daily would be taken either directly from ingested food or from tissue containing calcium in the creation of calcium oxalate.

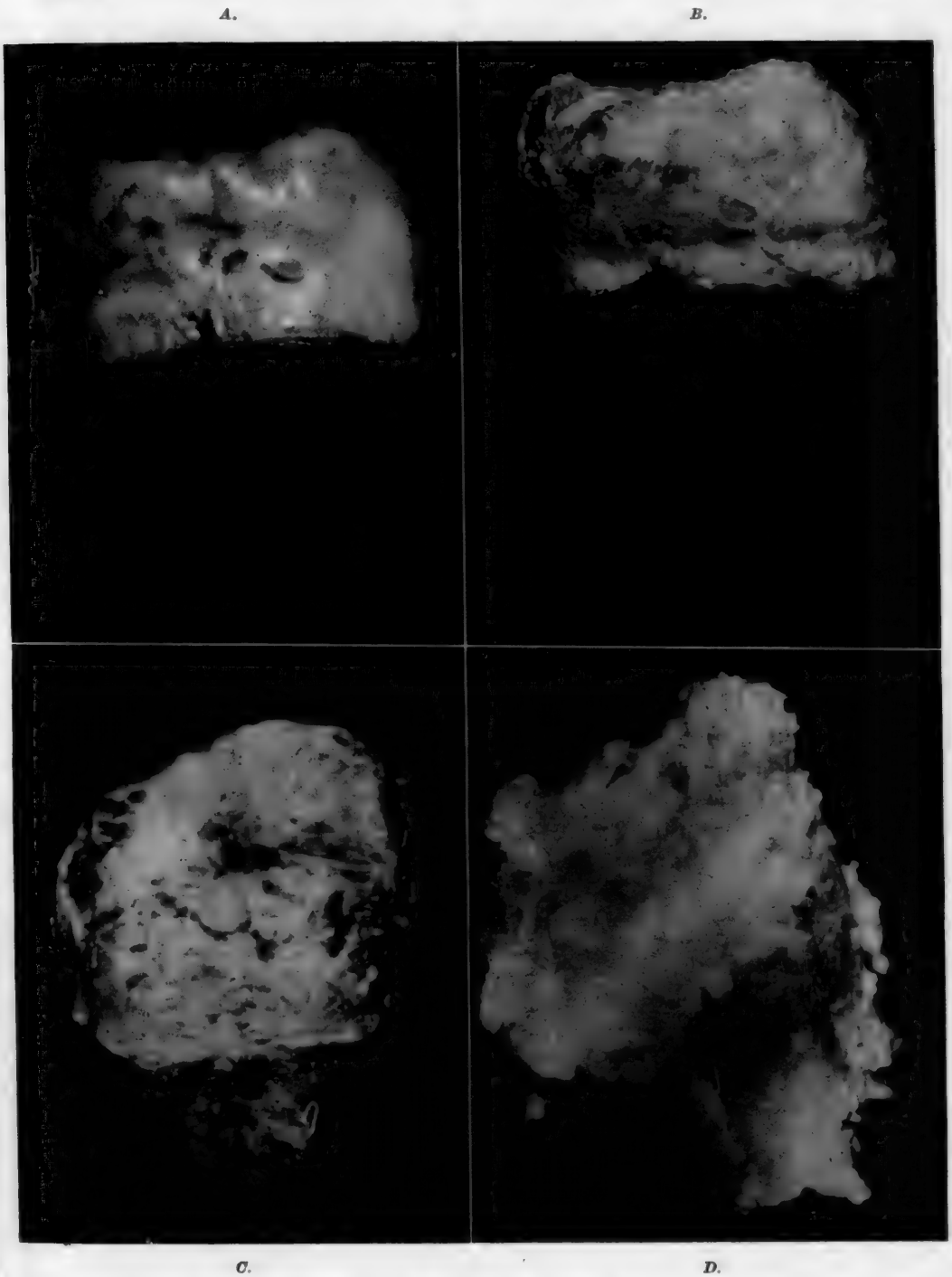

Fig. 1.—*A*. Tooth before immersion in saturated oxalic acid.
B. Same tooth 3 weeks after immersion in oxalic acid.
C. Same tooth 3 months after immersion in oxalic acid.
D. After 1 year in oxalic acid. Such structure as is left from tooth in *A*.

In order to investigate the possible effects of oxalic acid on such a calcified structure as tooth enamel, a tooth was placed in saturated oxalic acid; as might be expected, removal of calcium ions from the tooth structure (Fig. 1) was followed by the deposition of amorphous calcium oxalate over the crown area, and on the bottom of the glass container. A tooth in less concentrated oxalic acid showed a redeposition of material giving a knobby appearance over the crown of the tooth.

We suggest that for certain individuals with a particular type of amylase system, starch may be a most important source of oxalic acid. Subsequent interference with calcium metabolism may be of significance not only to the dentist treating an individual with severe dental caries,[14] but to physicians as well. The fact that the body protects itself from oxalic acid at the expense of calcium may result in a robbing of tooth calcium and food calcium while the relative insolubility of the resultant calcium oxalate in aqueous solution may account for the deposition of calcium oxalate in the ureters, arteries, or elsewhere.

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TERMINAL ALKALINITY IN SALIVA-PHENOL RED GLUCOSE BROTH CULTURES

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THE substitution of Phenol Red Glucose Broth (pH 7.4)* for acid glucose broth (pH 5.0) in Hadley's technic[1] for the quantitative estimation of numbers of lactobacilli in the saliva led to the following observation: after an initial acidity in 24 hours, reversion in 4 to 30 days to the alkaline side of the indicator was a fairly common occurrence. This shift was apparently caused by the enzymatic release from the organic nitrogenous substances in the medium of basic products which neutralized the acid already formed by the fermentation of the glucose. This process comes under a broad definition of "proteolysis" and, in view of recent controversies concerning the role of proteolysis in dental caries, it seemed of interest to investigate this problem of terminal alkalinity in saliva-glucose broth cultures. The present paper presents our findings in respect to the establishment of frequency of occurrence, the organisms, and factors responsible.

A. Frequency of Occurrence:

Since the original observations were made on discarded cultures, it was obvious that it would be necessary to keep the saliva-glucose broth cultures longer than the usual 48 hours. This period was arbitrarily set for 30 days at 37° Centigrade. The tubes in which changes appeared were easy to detect by a deepening of the yellow of the indicator, followed by a trace of orange, and finally by a return to the original pink-red color. Of 206 specimens of saliva received for routine culture in the Caries Diagnosis Laboratory Service,† 31 (15 per cent) showed terminal alkalinity. This represents a rather small series, but other observations from time to time would support this finding of 15 per cent of saliva-glucose broth cultures becoming alkaline within thirty days. Limited incubator space prevented storage of all saliva-glucose broth cultures made at this time.

B. Organism Causing Change:

As each positive color change was observed, a loopful of the broth culture was streaked over the surface of a blood agar plate and the components purified by further subculture. These forms were found to be staphylococci, lactobacilli, and yeastlike fungi. Streptococci seldom survived for thirty days in these cultures, as previously reported.[2] Except in one case, which was caused by *Pseudomonas aeruginosa (Bact. pyocyaneum)* only the yeastlike fungi were found capable of producing this reversion to the alkaline side of the indicator during the period of observation. It was apparent, however, that not every

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TABLE I

INFLUENCE OF COMPONENTS OF PHENOL RED GLUCOSE BROTH AT VARYING pH UPON THE ABILITY OF ORAL STRAINS OF CANDIDA TO PRODUCE A REVERSION TO THE ALKALINE SIDE OF THE INDICATOR

	NO. STRAINS	pH	0.5% PEPTONE 0.1% GLUCOSE 0.0% MEAT EXTRACT			0.0% PEPTONE 0.1% GLUCOSE 0.3% MEAT EXTRACT			PEPTONE 0.5% % GLUCOSE:													
									0.1			0.2			0.5			1.0			3.0		
DAYS OBSERVED			10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
C. albicans	29	7.0	6*	13	29	0	1	29	16	25	29	8	17	29	3	10	29	1	3	29	1	1	29
	29	6.0	4	10	29	4	5	20	14	26	29	9	21	29	2	5	28	0	3	29	0	1	28
	29	5.0	2	6	29	0	0	24	2	12	29	0	16	29	0	6	28	0	2	29	0	0	29
C. kruzei	3	7.0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	1	3	3
	3	6.0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	1	3	3
	3	5.0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	1	3	3

*Number of strains showing alkaline reaction at time of observation.

specimen of saliva containing these forms produced terminal alkalinity. Of 206 specimens, 52 (25 per cent) contained candida and 31 (15 per cent) showed terminal alkalinity. While yeastlike forms occur in about 25 per cent of specimens, a figure agreeing with that of Fisher[3] only 60 per cent of these strains produced terminal alkalinity in the cultures. However, separate studies under controlled conditions made it clear that the ability to produce this reversion is a uniform physiological feature for this group.

C. Identification:

According to Knighton and others[4, 5, 6] the yeastlike fungi occurring in the sputum and oral cavity belong almost without exception to the monilias which are now classified in the genus Candida. They are characterized by their growth on acid media as large creamy colonies which microscopically are composed of elliptical yeast cells showing the usual division by budding. Mycelia or pseudomycelia are produced upon starvation media, such as corn meal agar. Ascospores cannot be demonstrated, and they are active fermenters of carbohydrate in contrast to members of the family Rhodotorulaceae.[7]

For our studies we selected 31 strains of Candida from consecutive specimens of saliva sent to the laboratory. Six of these strains were known to produce terminal alkalinity. Based on morphological and physiological properties, 28 of the 31 strains fitted the description given for *C. albicans**; that is, mycelia were produced in corn meal agar, no ascospores could be demonstrated upon carrot plugs; and glucose, galactose, maltose, sucrose, and starch were fermented with acid and gas; lactose and mannite were not fermented. The other three strains which differed in growth and fermentative characteristics were identified as *C. kruzei.*

D. Influence of Medium:

Since it was assumed that this change was the resultant of basic products formed either from the nitrogenous constituents of the medium or, possibly, the cells themselves, each component of the medium was tested for its ability to influence this reaction. The results obtained with 29 strains of *C. albicans* (a known strain added to the original 28) and 3 strains of *C. kruzei* under varying hydrogen-ion concentrations at the time of inoculation are given in Table I.

Analysis of Table I shows clearly the effect of the concentration of glucose in the appearance of terminal alkalinity in nutrient broth by both species, although the final results were not significantly altered by either the presence of 3 per cent glucose or decreasing pH. Similarly, the rate of alkaline production was greater with both meat extract and peptone than with either component. Lowering of the pH also prolonged in general the time before an alkaline reaction was observed, although with the lowest concentrations of glucose and in the absence of peptone, alkaline reactions appeared more rapidly at pH 6.0 than pH 7.0 or pH 5.0. We have made similar observations in other experiments, but can offer no explanation for this peak.

*Confirmed for a representative strain by Dr. Norman S. Conant, Duke University, Durham, N. C.

Otherwise, the results are in accord with our knowledge concerning the alkalinization of cultures of bacteria when the glucose has been exhausted and the pH of the fermentation not too adverse for the proteolytic enzymes concerned.

DISCUSSION

The explanation for the original observation of the terminal alkalinity in Phenol Red Glucose Broth by microorganisms of the saliva was, therefore, the ability of oral strains of the genus Candida to produce this reaction under the growth conditions imposed. When this reaction was analyzed, it was clear that the most important factor was the concentration of glucose which in the original 0.5 per cent concentration allowed a more rapid return to the alkaline side of the indicator than with larger quantities of glucose. This rate could be further accelerated by reducing the amount of glucose to 0.1 per cent.

This type of reaction by the yeastlike forms in the saliva is in no way different than similar results obtained with many bacterial species in culture media low in glucose content. In these, the glucose is utilized first but the accompanying pH change is not sufficient to prevent proteolytic enzymes releasing substances from the medium which neutralize and then turn the culture alkaline.

Although a satisfactory answer for the original problem has readily been obtained, it is quite another matter to ascertain if this process in vivo would have any significance in either the concept of neutralization of acid production, and hence a defense mechanism in caries activity as postulated for *Aerobacter aerogenes* by Kesel, O'Donnell, Kirch, and Wach,[8] or in the proteolytic theory of dental caries.[9, 10] Studies to test some of these possibilities are now being conducted in this laboratory.

SUMMARY

The production of terminal alkalinity in Phenol Red Glucose Broth by specimens of saliva held at incubator temperatures for thirty days was caused almost entirely by oral strains of the genus Candida.

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THE ASCORBIC ACID CONTENT OF THE SALIVA OF CARIOUS AND NONCARIOUS INDIVIDUALS*

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HANKE, using orange juice as the source of ascorbic acid, suggested that it had therapeutic value on dental and periodontal tissue.[1] With the commercial synthesis of pure ascorbic acid, Grandison, Stott, and Quickshank repeated the work of Hanke on 20 children, 4 to 14 years, using 200 mg. ascorbic acid per child per day for 2 years, but found no improvement in their dental health, though their general health did improve.[2] Much of the later literature on this subject has emphasized the importance of ascorbic acid for general health improvement rather than for the prevention or decrease of the onset of dental caries.[2, 3]

Stuteville determined the level of vitamin C in saliva to be 0.25 mg. per cent, using the 2,6-dichlorophenolindophenol titration method.[4] Zimmet and Dubois-Ferrière repeated the work of Stuteville and found the level of vitamin C in the saliva of carious patients to be more than 50 per cent lower than that of normal noncarious patients.[5, 6] They also found that the administration of ascorbic acid does not affect it.[5] However, they report that the vitamin C content differs with age, 0.038 mg. per cent at 4 years, and then increases fairly rapidly till it reaches 0.101 mg. per cent at 16 years, and then slowly rises to 0.130 mg. per cent at 27 years, after which it remains constant.[7]

The relationship between the ascorbic acid content of the salivas of carious and noncarious persons reported by Zimmet and Dubois-Ferrière has not been tested by other investigators. At low concentrations of ascorbic acid, such as are found in the saliva, the end point in the indophenol titration method is difficult to judge, making accurate estimations highly improbable. We have undertaken to estimate salivary ascorbic acid using the colorimetric method of Roe and Keuther,[8] which has a high degree of specificity and is sensitive to the small amounts found by others in saliva. With the Roe and Keuther method, accurate and reproducible results could be obtained with saliva, and the present paper reports a series of studies on the ascorbic acid content of the salivas of normal and carious individuals.

EXPERIMENTAL

Reagents:

1. Ten per cent trichloroacetic acid: Dissolve 50 Gm. trichloroacetic acid in 100 ml. water. This is the stock solution. Then, dilute 20 ml. of this to 100 ml. for the working solution. The stock solution is stable if kept in a cold place, but the working (10 per cent) solution should be relatively freshly prepared.

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2. Ten per cent thiourea: Dissolve 10 Gm. thiourea in 100 ml. of 50 per cent (by volume) aqueous ethyl alcohol.

3. One per cent 2.4-dinitrophenylhydrazine: A. Prepare a 9 N H_2SO_4 solution by dissolving 26 ml. H_2SO_4 sp. gr. 1.84, in 100 ml. of water. B. Dissolve 1 Gm. 2,4-dinitrophenylhydrazine in 100 ml. of the above 9 N H_2SO_4 solution.

4. Eighty-five per cent H_2SO_4: Add 90 ml. concentrated H_2SO_4 to 10 ml. water.

5. Norite: Wash norite with a hot 10 per cent HCl solution till the washings give no color for iron with KSCN. After the test for iron is negative, wash the norite with distilled water and let dry in the air.

6. Standard solution: Dissolve 20 mg. ascorbic acid in 10 ml. 10 per cent trichloroacetic acid for stock solution, and dilute this 1 to 100 with 10 per cent trichloroacetic acid, and use 1 ml. of it as the standard. The standard then contains 0.020 mg. per milliliter.

METHOD

1. Into 10 ml. of a 10 per cent trichloroacetic acid put 10 ml. saliva and add about 0.50 Gm. acid-washed norite, shake well, and filter.

2. Pipette 4 ml. of this filtrate, which is equivalent to 2 ml. saliva, into a test tube. Add one drop of 10 per cent thiourea solution, and 1 ml. of the 1 per cent 2,4-dinitrophenylhydrazine reagent. Mix well and incubate for three hours at 37° C. Do this in duplicate.

3. Run a blank and a standard with each set of tubes by placing 2 ml. of a 10 per cent trichloroacetic acid and 2 ml. of water for the blank in a test tube, and 1 ml. 10 per cent trichloroacetic acid and 1 ml. dilute standard solution which is equivalent to 0.020 mg. ascorbic acid, and 2 ml. water for the standard in a second test tube. Add one drop thiourea, and one ml. 1 per cent 2,4-dinitrophenylhydrazine reagent to each tube: shake and incubate along with the unknown tubes for three hours at 37° C.

4. Remove unknown, blank and standard test tubes, and place in ice-water bath, and add, slowly, and from a burette, into each tube 5 ml. of the 85 per cent H_2SO_4. Mix well, and keep in a rack for one-half hour at room temperature.

5. Read in a Klett-Summerson photoelectric colorimeter, the blank, standard, and unknown tubes, and calculate: $\frac{\text{Unknown minus blank}}{\text{Standard minus blank}} = \text{mg. per cent ascorbic acid in saliva.}$ Filter Number 42 should be used in the colorimeter.

DISCUSSION

In Table I are given the values for the determinations of ascorbic acid in the unstimulated saliva of 31 individuals free from caries and without dental restorations and missing teeth,* while in Table II are given the values for 54 carious individuals. The analyses were performed, in almost all of the cases, on saliva collected two hours after breakfast. We did collect saliva from the same individual at other times of the day, always several hours after eating, and found no significant variation in ascorbic acid content, confirming the results of Zimmet and Dubois-Ferrière.[5] We also ran several saturation tests by administering 500 mg. ascorbic acid dissolved in about 400 ml. of water. Ascorbic acid was determined in the saliva at zero time, half-hour, and hourly for three hours. Again, we confirmed the results of the above named investigators that there was no significant variation in the ascorbic acid level of the saliva.[5]

*The carious individuals were examined under the supervision of Joseph J. Kelly, D.D.S., Professor of Operative Dentistry.

TABLE I

ASCORBIC ACID LEVEL IN THE SALIVA OF NONCARIOUS PATIENTS

NUMBER	AGE*	ASCORBIC ACID MG. %
1	xx	0.20
2	xx	0.11
3	xx	0.31
4	xx	0.14
5	19	0.19
6	xx	0.21
7	xx	0.33
8	xx	0.25
9	xx	0.22
10	xx	0.10
11	19	0.28
12	xx	0.26
13	18	0.24
14	xx	0.36
15	xx	0.28
16	12	0.22
17	xx	0.37
18	11	0.17
19	13	0.10
20	15	0.13
21	16	0.15
22	xx	0.33
23	xx	0.31
24	xx	0.13
25	xx	0.29
26	9	0.10
27	9	0.12
28	7	0.21
29	xx	0.08
30	xx	0.23
31	xx	0.26
Average		0.218 ± 0.080

*xx stands for above 19 years of age.

The average ascorbic acid content of the saliva of the normals was 0.218 mg. per cent. When this was broken down to three age groups, Table III, the differences in the averages of the three groups from the general average were not significant. When the averages of the ascorbic acid value for carious individuals was similarly broken down, again there was no significant difference, nor was there a difference when the values were related to the number of carious surfaces. While the average ascorbic acid content of the saliva from the carious individuals was slightly below the average for those without caries, no statistical significance could be attached to the difference.

TABLE II

ASCORBIC ACID LEVEL IN THE SALIVA OF CARIOUS PATIENTS

NUMBER	AGE*	NUMBER OF CARIOUS SURFACES	ASCORBIC ACID MG. %
1	14	5	0.19
2	16	10	0.09
3	xx	10	0.13
4	15	40	0.20
5	xx	3	0.22
6	16	15	0.21
7	xx	5	0.15
8	9	5	0.09
9	13	14	0.11
10	16	14	0.12
11	18	20	0.13
12	19	9	0.19
13	18	14	0.12
14	xx	21	0.18
15	xx	17	0.09
16	xx	21	0.15
17	xx	19	0.33
18	7	17	0.19
19	xx	10	0.21
20	19	9	0.20
21	11	6	0.31
22	xx	29	0.13
23	xx	9	0.31
24	19	14	0.22
25	19	29	0.09
26	xx	7	0.12
27	17	12	0.16
28	xx	13	0.28
29	xx	5	0.15
30	15	17	0.17
31	16	40	0.23
32	7	10	0.22
33	8	4	0.31
34	15	5	0.11
35	14	6	0.13
36	13	21	0.20
37	xx	5	0.32
38	13	8	0.10
39	8	4	0.20
40	10	2	0.16
41	8	9	0.10
42	6	3	0.19
43	17	3	0.20
44	xx	5	0.20
45	xx	14	0.10
46	9	8	0.10
47	5	10	0.23
48	16	5	0.19
49	8	15	0.18
50	15	16	0.10
51	xx	3	0.10
52	12	20	0.19
53	12	20	0.21
54	10	21	0.17
Average			0.176 ± 0.063

*xx stands for above 19 years of age.

TABLE III

LEVEL OF ASCORBIC ACID IN SALIVA OF CARIOUS AND NONCARIOUS PATIENTS

A. Carious Cases: *1. Classified according to age:*

NUMBER	AGE	RANGE MG. %	AVERAGE MG. %
15	4 to 12	0.09 to 0.13	0.195
22	13 to 19	0.09 to 0.23	0.152
17	20 and up	0.09 to 0.33	0.190
Total 54			

2. Classified according to carious surfaces:

NUMBER	CARIOUS SURFACES	RANGE MG. %	AVERAGE MG. %
29	1 to 10	0.09 to 0.32	0.180
14	11 to 19	0.08 to 0.33	0.173
11	20 and up	0.09 to 0.23	0.171
Total 54			

B. Noncarious Patients:

NUMBER	AGE	RANGE MG. %	AVERAGE MG. %
5	7 to 12	0.10 to 0.22	0.164
6	13 to 19	0.10 to 0.28	0.218
20	20 and up	0.08 to 0.37	0.219
Total 31			

CONCLUSIONS

Ascorbic acid was determined in saliva by the colorimetric method of Roe and Keuther.[8] The average value for 31 noncarious individuals was 0.21 mg. per cent, and for 54 individuals with caries was 0.17 mg. per cent. No statistical significance can be attached to the difference, nor could any correlation be found between ascorbic acid content and age, or the number of carious surfaces.

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THE CHEMICAL NATURE OF THE PROTEINS FROM HUMAN ENAMEL

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A KNOWLEDGE of the composition of enamel is essential to an understanding of the processes of dental caries. While considerable work has been done on the inorganic portion of enamel, less attention has been given to the organic part. This paper describes some experiments on the isolation of the enamel proteins and the determination of their cystine, methionine, and phenylalanine content.

Karlstrom, from micro-Kjeldahl determinations, calculated that the protein content of enamel ranged from 0.1 to 0.66 per cent with an average value of 0.3 per cent.[1] Sprawson, using enamel filings, found 0.15 per cent protein based on nitrogen determinations, and from carbon analysis, 0.18 to 0.21 per cent protein.[2] Rosebury and Gies isolated enamel protein by dialysis and reported 0.3 to 0.54 per cent in human enamel.[3] These investigators recognized the presence of a surface cuticle and an inner layer, and attempted to remove both prior to isolation of the protein. Armstrong and Brekhus determined the nitrogen content of the enamel from four normal teeth to be 0.098 per cent equivalent to not more than 0.60 per cent protein.[4] LeFevre and Manly employed a semiquantitative method for the determination of the organic content of enamel.[5] They deducted the moisture and inorganic content from the original weight of the enamel and found from 0.6 to 2.9 per cent, with an average value of 1.7 per cent total organic material. Deakins and Volker determined the total nitrogen content of enamel from rat teeth and calculated the protein content to be 2.97 per cent, while the enamel from old noncarious permanent human teeth gave a value of 1.75 per cent protein.[6] It is of interest to note that they found, by this method, 0.49 per cent protein in the enamel of carious, young, permanent human teeth.

Since the amount of protein in enamel is quite small it is not surprising that very few investigators have attempted to study its amino acid content. Pincus was unable to find any sulfur in enamel protein and stated that it is a scleroprotein resembling reticulin.[7] He based the latter statement of the finding that it is resistant to enzymatic hydrolysis. Pincus gives no quantitative data nor does he state the amount of protein isolated or tested. Kanner was unable to find cystine in an hydrolysate of enamel protein by the polarographic method of Heyrovsky.[8] Schoeller, as reported by Pincus, found from one to two per cent sulfur in enamel protein.[9] More recently Pincus has reported the isolation of what he calls "enamel protein" but which should more properly be termed

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cuticle protein since it was removed from the surface rather than the body of the enamel.[10] He found tyrosine in the material and stated that it resists enzyme attack. Neither Kanner[8] nor Pincus[10] gave any quantitative data.

EXPERIMENTAL

Teeth.—The teeth employed in this investigation were freshly extracted, human, noncarious, erupted permanent molars. Some were chosen for the presence of the acquired cuticle or brown pigmented recurrent pellicle referred to by Bibby[11] and Manly.[12]

The enamel crown was defined as containing three component parts:

1. The enamel cuticle: The surface or outer layer that remains after a careful cleaning procedure designed to remove any acquired cuticle or pellicle. No attempt is made to differentiate it into primary or secondary enamel cuticle or both.

2. The enamel proper: The middle or main body of the enamel that would contain the prisms, prism sheaths, interprismatic substance, lamellae, and pseudolamellae.

3. The inner enamel: The layer including the area immediately above the dentino-enamel junction. This layer includes the gnarled prisms, enamel tufts, enamel spindles, dentinal processes, lamellae, and pseudolamellae. It comprises approximately the inner one-fourth of the enamel.

An effort was made to choose the teeth and prepare the enamel so that these various enamel components could be most effectively studied. Since the inner enamel contains dentinal components and is affected last by the carious process, it was removed from the enamel of all the teeth. Five types of material were selected for analysis: (1) total enamel, cuticle + enamel proper, with minimal pits and grooves and unstained; (2) total enamel with deep pits and grooves and unstained; (3) total enamel with deep pits and grooves and stained; (4) enamel proper from teeth of the same morphology and color as Category 1; (5) enamel cuticle that was removed from samples of Category 1.

Methods of preparation of the samples.—The selected teeth were first scrubbed thoroughly with a surgeon's handbrush and castile soap. All soft tissue was removed and a second brushing was given with a hard-bristled nylon toothbrush. This was followed by a motor-driven bristle brush and a rubber cup with fine pumice and water. Care was taken to prevent the generation of excess heat, and not over three minutes was devoted to this step. The crowns were then immersed in 5 per cent hydrochloric acid for thirty seconds as recommended by Rosebury to remove Nasymyth's membrane and debris.[13] It must be noted, however, that it is our experience that this procedure does not completely remove the true enamel cuticle. The crown was again scrubbed with a toothbrush and castile soap and rinsed.

The crown was then placed into a plaster of Paris mound to give lateral support to the walls while the dentin was ground out. The removal of the dentin was accomplished with round steel burs, progressively decreasing in size, to assure it removal from the tip of the dentinal cusp. The inner layer of enamel

was removed with a diamond stone. After removal from the plaster, the crown was scrubbed free of adhering plaster and air dried, then placed in a quartz mortar and pulverized. Sample 3 with the brown pigmented pellicle was prepared in the same manner, with the exception of the acid bath.

The enamel proper and the enamel cuticle were obtained by placing the dentin-free crown, that had been cleaned by the above procedure, into a Stender dish for an additional bath with 5 per cent hydrochloric acid, under a dissecting microscope. In three to five minutes the enamel cuticle could be teased off with a dissecting needle, and separated. The average sample contained 13 grams of enamel from approximately 27 crowns; each crown required from fifty-five to sixty-five minutes for its preparation.

Demineralization.—The dialytic demineralization procedure of Rosebury was modified so that larger samples of enamel could be processed in a comparatively short time.[13] The flotation process of Manly and Hodge was not used because it was felt that the difference in density between some of the enamel particles and the particles of dentin was not sufficiently large to ensure complete recovery of pure enamel.[14] Further, the possibility of a reaction of the flotation liquid upon the enamel protein was eliminated. The powdered enamel was placed in a Pyrex glass tube 75 mm. long and 40 mm. in diameter, this tube was covered at both ends with two sheets of Cellophane to serve as the semipermeable membrane. The tube was placed into a two-liter beaker and attached to an agitation arm by tying the two ends in such a manner as to cause a mixed horizontal and vertical movement. The beaker contained 1.5 liters of a 2 per cent acetic acid solution, which was changed every twenty-four hours. After agitation for four or five days there seemed to be no further reduction in bulk, and at this time 2 per cent hydrochloric acid was substituted for the acetic acid. The demineralization was considered complete when the molybdate test for the phosphate ion in the external fluid was negative for three consecutive days.

Soluble protein.—The external fluid was tested daily for any soluble and diffusible protein using the Millon's, xanthoproteic acid, Hopkins-Cole, and biuret color tests. All the tests were negative indicating that no soluble protein or hydrolytic products were present.

Insoluble protein.—The residue remaining in the dialysis cell, after demineralization was complete, was washed into 50-c.c. centrifuge tubes. After centrifugation the supernatant fluid was tested for the presence of soluble protein or partial decomposition products of the insoluble protein by the same color tests used upon the external fluid; none of the tests were positive. The precipitate was washed and centrifuged successively with 70 per cent, 95 per cent, absolute ethyl alcohol, 1:1 absolute alcohol and ether, and finally with ether. The protein residue was dried in a desiccator over calcium chloride.

Quantitative determination.—Moisture and Ash. Air-equilibrated powdered enamel from crowns with deep pits and grooves but unstained was weighed and placed in a desiccator over calcium chloride until the weight was constant. After ten days the moisture content, by this procedure was 0.114 per cent.

deep pits and grooves. In addition, deep pits and grooves have been removed from other crowns by grinding and then the proteins were isolated. The protein content, 0.49 per cent, was highest in the total enamel from stained crowns with deep pits and grooves and lowest, 0.30 per cent, from unstained crowns with minimal pits and grooves. The enamel cuticle was 0.08 per cent of the total weight of the crown. This value, when added to the 0.22 per cent protein isolated from the same crowns from which the cuticle had been removed, gave 0.30 per cent protein, a value identical with that obtained from the total enamel from similar crowns.

An attempt was made to calculate the protein content of the enamel from the total nitrogen determinations. Using the factor 6.25, which is probably too low, a value of 0.46 per cent protein was obtained from crowns with deep

Erratum

The contents of pages 515 and 517 were transposed on the press. Please read 514, 517, 516, 515.

Correction is made in this manner rather than by reprinting forms completely bound to avoid delay in publication.

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TABLE I

YIELDS OF PROTEIN FROM THE SEVERAL TYPES OF ENAMEL

MATERIAL ANALYZED	WEIGHT OF ENAMEL GM.	WEIGHT OF PROTEIN GM.	PROTEIN %
1. Total enamel with minimal pits and grooves, unstained	9.58	28.3	0.30
2. Total enamel with deep pits and grooves, unstained	12.60	48.6	0.39
3. Total enamel with deep pits and grooves and stained	13.3674	65.9	0.49
4a. Enamel proper with minimal pits and grooves	8.249	17.7	0.22
b. Enamel proper with pits and grooves ground out	4.2045	8.60	0.21
5a. Enamel cuticle from crowns with minimal pits and grooves	13.367	10.7	0.08
b. Enamel cuticle from crowns with pits and grooves ground out	13.40	6.10	0.05

TABLE II

CYSTINE, METHIONINE, AND PHENYLALANINE CONTENT OF THE SEVERAL ENAMEL PROTEINS

SOURCE OF PROTEIN	CYSTINE %	METHIONINE %	PHENYLALANINE %
1a. Total enamel with minimal pits and grooves, unstained	0.60	1.01	6.2
b.* Total enamel	0.36		
2. Total enamel with deep pits and grooves, unstained	0.76	0.97	5.8
3. Total enamel with deep pits and grooves, stained	1.00	1.19	
4a. Enamel proper with minimal pits and grooves	0.20	1.74	4.6
b.* Enamel proper	0.17	1.54	
5. Enamel cuticle from crowns with minimal pits and grooves	0.94	1.11	5.1

*b = Pits and grooves ground out.

The proteins from the five types of material previously described are given in the first column. In several instances proteins were prepared from the same type of enamel except that the deep pits and grooves were ground out, and these are designated as the (b) samples. It is of interest to note that the enamel cuticle has the highest cystine content, while the protein isolated from the enamel freed from cuticle has the highest methionine content. The phenylalanine content, in common with most protein, is fairly high in all the samples. The proteins from all the fractions do contain cystine but in amounts far less than are found in true keratins such as hair and nails. Until analyses for histidine, lysine, and arginine are made, it will not be possible to determine if these proteins are true or pseudo-keratins according to the criteria set up by Block.[18]

SUMMARY

Enamel, from human, freshly extracted, noncarious, erupted permanent molars, has been divided into several parts, the cuticle, the enamel proper, and the total enamel, comprising both the cuticle and the enamel proper. Proteins have been isolated from these three divisions, from crowns with minimal pits and grooves and unstained, also from both stained and unstained crowns with

Another sample dried to constant weight in an oven at 110° C. gave a moisture content of 0.699 per cent. The oven-dried sample ashed with a Fisher burner for 4 hours gave an ash content of 96.07 per cent. It was noted that further heating produced additional loss in weight. After thirty hours' heating with the Fisher burner, the weight of the ash remained constant within 0.1 mg. and the new ash value was 95.19 per cent. During the process of ashing, carbon dioxide is lost from the inorganic portion of the enamel. According to Deakins and Volker, this loss ranges from 3.0 to 4.0 per cent.[6] As an approximation, the organic content of the enamel can be estimated by adding the amounts of carbon dioxide and moisture lost to the ash content and subtracting from 100 per cent. Upon this basis the possible organic content of the enamel is from 0.2 to 1.1 per cent. Determinations of total nitrogen by the Kjeldahl method, upon duplicate 500 mg. samples of total powdered enamel, gave a value of 0.073 per cent nitrogen. If this figure is multiplied by the usual factor 6.25, a possible protein value of 0.46 per cent is obtained. The uncertainty in the actual loss of carbon dioxide during ashing prohibits a close correlation between the two methods of estimation.

The actual amounts of protein isolated by the dialytic determination procedure are given in Table I. It can be seen that from the total enamel with deep pits and grooves (2), 0.39 per cent protein was isolated, while from the total enamel with minimal pits and grooves, (1), 0.30 per cent protein was isolated. When the enamel was stained (3), the per cent protein isolated was even higher, 0.49 per cent. From the enamel proper (4), the yield was 0.22 per cent, and the amount of cuticle (5, a.) isolated from the same enamel was 0.08 per cent. The sum of these two is 0.30 per cent, identical with the value from (1), the total enamel with minimal pits and grooves and unstained. The amount of protein isolated depends upon the character of the enamel studied, the deeper the pits and grooves the higher the protein content, probably because of a higher cuticle content in these areas. When the deep pits and grooves were ground out, the amount of protein isolated approximated that from the crowns with minimal pits and grooves. The protein value calculated from the total nitrogen is 0.46 per cent, this value is probably low since the factor 6.25 is based upon nitrogen content of 16 per cent which may be too low according to results published by Pincus.[9] However, it is apparent that the amount of protein isolated is within the range established by the nitrogen values. Further studies are contemplated upon the nitrogen content of the isolated proteins.

Cystine, methionine, and phenylalanine.—The fat-free insoluble protein was hydrolyzed with 20 per cent hydrochloride acid in 50-c.c. acetylation flask fitted with a ground glass reflux condenser. The hydrolysis was carried out by partially immersing the flask in a wax bath maintained at 125° C. for a period of six hours. The cystine method of Hess and Sullivan,[15] the modified McCarthy-Sullivan[16] methionine method of Hess and Sullivan,[15] and the phenylalanine method of Hess and Sullivan[17] were employed upon the hydrolysate. All the colorimetric readings were made with a Klett-Summerson photoelectric colorimeter using a 42 filter. The results are given in Table II.

COMPARATIVE PROTEIN CHEMISTRY

The Composition of the Proteins of Human Teeth and Fish Scales

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THROUGHOUT almost the entire vertebrate kingdom the various calcareous or horny structures serve for defense, support of tissues, or attachment of muscles. Histological investigations have shown that the horny tissues originate in the stratified squamous epithelium and become more highly keratinized as they are pushed farther and farther away from their sources of nourishment. Comparative biochemical studies have shown that the principal proteins of horny structures have certain properties in common which permit them to be classified together. These proteins, keratins, are resistant to digestion, insoluble in dilute acids and alkalies, water and organic solvents.[1] Keratins which are chemically similar to cattle horn (*Κερας*) have been called eukeratins. Eukeratins are defined as keratins which yield histidine, lysine, and arginine in the molecular ratios of approximately 1:4:12. Those keratins which do not yield the basic amino acids in molecular ratios of approximately 1:4:12 have been designated pseudokeratins.[2, 3] The ash content of these keratins is usually less than 10 per cent.

Vertebrates also employ calcified collagens for defense, support of tissues, or attachment of muscles. Collagens are converted by boiling with water into easily digestible soluble proteins, gelatins. Chemically the collagens may be characterized by their content of hydroxyproline which has not been proved to occur in many other proteins of vertebrate origin.[3]

Among vertebrates the placoid scales are important because of their further evolution. Each of these scales has a flat basal plate of dentin embedded in the skin, and each commonly has a projecting spine coated with hard enamel.* From these minute placoid scales of ancient sharks, the multiform "true" teeth

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*Placoid scales (denticles) from sand sharks were found to contain 3.3 per cent of nitrogen and 70.5 per cent of ignition residue (CaO and SiO_2) as received. When the denticles, either before or after decalcification with acid, were boiled with water, more than two-thirds of the nitrogen was obtained as gelatin. The insoluble residue (2.8 Gm.) was then digested with 30 mg. of 1:10,000 pepsin in 1 liter of 0.36 per cent of HCl at 40° C. for 3 days. The precipitate which remained was then removed and washed with water and dried with alcohol. This residue was digested with 100 mg. of Pancreatin in 500 ml. of 0.1 M phosphate buffer of pH 8.0 for 3 days. Fifty-five ml. of isopropanol were used as the preservative. At the end of the pancreatic digestion, the residue was removed, washed with water, alcohol and ether, and dried in the air. The yield of shark "keratin" was approximately 0.4 per cent based on the weight of the orginal placoid scales. N = 11.6 per cent. The molecular ratio of histidine to lysine to arginine in this residue was 1:4:4, whch indicates that it is not an eukeratin.[1] The following amino acids were seen on the two-dimensional paper chromatogram: glutamic acid, glycine, serine, alanine, and threonine gave strong spots. Leucine, phenylalanine, isoleucine, methionine, valine tyrosine arginine, and lysine gave spots of medium intensity. Weak spots were given by proline, histidine, and cystine. Hydroxyproline was not seen. It appears, therefore, that the placoid scales of sharks probably do not contain eukeratins but consist of minerals, collagens, and possibly pseudokeratins.[2, 3]

of all the higher vertebrates are believed to have evolved. It is also commonly accepted that the dermal armor of the ganoids and other fishes has evolved from the placoid scales of the elasmobranchs by simple enlargement.[4] The development of true teeth is believed to be similar to that of placoid scales; in both the enamel is secreted by the ectoderm and the dentin by the mesenchyme.

Dental enamel is recognized as the hardest and most completely mineralized tissue in the human body. It contains approximately 0.4 per cent of insoluble protein.[5] Hoppe appears to have been the first to suggest that the protein of dental enamel is similar to that of the epithelial tissues.[6] Thompson in 1887[7] referred to the organic material of enamel as "keratine." Rosebury,[5] because of the low sulfur content of enamel protein, thought that it was more closely related to neurokeratin than to the keratin of the integument. Kanner,[8] using the sensitive polarographic method, was unable to detect any cystine in enamel protein after hydrolysis and, therefore, questioned the classification of this tissue protein as a keratin. Likewise, Pincus,[9, 10] on the basis of low sulfur content and extreme resistance to enzymatic digestion, was reluctant to place it either as a eukeratin or a pseudokeratin. When our experimental investigations were almost completed, a paper by Pincus[11] appeared. Pincus studied the proteins on the surface of unerupted human molar teeth. He said, "Human dental enamel protein has been shown to consist of two proteins, Nasmyth's membrane and groove protein." The latter protein gives many characteristics of a mucoprotein. As the majority of previous investigations and our own studies were carried out on the protein prepared by decalcification of the enamel of erupted teeth from which any Nasmyth's membrane had been removed, the classification of Nasmyth's membrane and groove protein as "human dental enamel protein" is, we believe, unwarranted at least at the present time.

In view of the doubt as to the classification of human dental enamel protein, it was deemed of interest to study the amino acid composition of human enamel proteins, human dentin protein, and the scales of the herring, *Clupea harengus*. The results indicate that the protein of human dental enamel is an eukeratin. By the same criteria, human dentin protein is a typical collagen, while the proteins of herring scales may be divided into two groups of proteins which are present in approximately equal proportions. One is a collagen, while the second, although it is not converted into gelatin on boiling with water, contains hydroxyproline, is easily digestible by pepsin and by trypsin, and may be assumed to be an albuminoid of the collagen type (ichthyledpidin, 12).

Manly[13] reported a structureless recurrent brown deposit on the labial surfaces of the anterior teeth of some individuals who do not use abrasives in their oral hygiene. As a result of carefully conducted experiments on the minute quantities of material which were available to him, Manly concluded that the "brown pellicle" should be classed as a keratin. On the other hand, Vallotton,[14] largely on the basis of negative evidence, believed it to be a "denatured or otherwise altered mucin." Manly's suggestion[13] that the "brown pellicle" may be a keratin prompted us to compare the properties of this material with those of human enamel keratin. As a result of the investigations herein reported, it is concluded that "brown pellicle" is not an eukeratin.

EXPERIMENTAL

Preparations

Human Enamel Proteins.—Dentin was removed from the enamel of erupted human teeth by abrasion followed by the bromoform floatation method of Manly and Hodge.[15] The resulting pure enamel was extracted with cold 5 per cent HNO_3 to remove ash and the acid removed by washing with water. This material, 86.7 mg., was ground and then thoroughly extracted with hot acetone, hot benzene, and alcohol-ether (3:1) successfully. The residue was digested at 37° C. with 5 mg. of 1:10,000 pepsin in 0.1 N HCl for 42 hours, the residue was washed 3 times with 40 ml. portions of water, and then digested with 10 mg. of crystalline chymotrypsin at pH 7.7 for 90 hours. The insoluble residue from this digestion was washed three times with water, extracted with the alcohol-ether mixture and dried in vacuo. The yield of enamel keratin was 31.2 mg. N = 11.9 per cent.

Human Dentin.—A sample of dentin from extracted erupted human teeth was given to us by Dr. Walter C. Hess of Georgetown University Medical School, Washington, D. C. This material (77 mg.) was converted into gelatin by boiling with water to 15 minutes. The slight amount of insoluble residue (2.4 mg.) was removed by filtration through a sintered glass crucible. The filtrate was evaporated to dryness, yield 75 mg. N = 15.8 per cent.

"Brown Pellicle."—The brown film described by Manly[13] was collected, with the assistance of a number of dentists, from persons who were either not brushing their teeth or who were using a nonabrasive dentifrice. In order not to confuse the film under investigation with the brown stain found in tobacco smokers, only nonsmokers were examined. Over three hundred nonsmoking female patients were observed, of whom only about 10 per cent showed Manly's brown pellicle on their teeth. Later, when men patients were employed, it was observed by the dentists that the brown pellicle occurs more frequently in male patients than in female patients. The brown pellicle, which forms on the teeth of males, was softer and more easily removed from the teeth. In all, over one thousand nonsmoking patients were examined of whom approximately 100 were suitable as a source of supply of the pellicle.

The teeth were brushed with water or a nonabrasive dentifrice in order to remove extraneous material. Then, the brown pellicle was removed from the labial surfaces of the anterior teeth with the aid of a scraper. This material was preserved in chloroform.

The pooled crude brown pellicle material collected from women during the period 1945-1946 weighed (wet) 112 mg.; 71.7 mg. of this material were extracted with 15 ml. of 0.11 N HCl at 25° C. overnight. The residue was then washed with water and treated with 5 mg. of 1:10,000 pepsin (Wilson) in 20 ml. of 0.05 N HCl at 37° C. for 18 hours. The residue from the peptic digest was removed, washed, and treated with 5 mg. of chymotrypsin (crystalline) in 20 ml. of M/30 phosphate buffer at pH 7.7. The yield of brown pellicle after digestion was 12.0 mg. after washing and drying.

The 1946-1947 collection of brown pellicle was more successful; 168 mg. of crude material from both men and women were extracted with organic solvents, and the residue was digested with pepsin HCl for 8 days, then with trypsin for 7 days, and with chymotrypsin for 6 days. The residue from the digestions was thoroughly washed with water and dried with alcohol and acetone. The yield was 25.8 mg. N = 11.8 per cent.

Gelatin and Ichthylepidin from Herring Scales.—Air-dried herring scales were washed with water and gasoline to remove dirt and guanine. The air-dried scales (nitrogen = 9.44 per cent, ash 41 per cent calculated as $CaCO_3$) were analyzed as such. The fish scales, either with or without the removal of the $CaCO_3$ with SO_2 or dilute HNO_3, were readily digested by pepsin and trypsin.

Separation of herring-scale proteins into gelatin and ichthylepidin was accomplished as follows: 55 Gm. of scales were suspended in 500 ml. of water and SO_2 was passed into the stirred solution to pH 2.0. The insoluble residue was removed by filtration. The clear filtrate contained a large quantity of $CaHSO_3$ but little, if any, protein as indicated by the usual tests. The insoluble residue was air dried and then suspended in 600 ml. of water. The collagen was converted into gelatin by maintaining at 80 ± 5° C. for 90 minutes. The insoluble residue was removed and extracted with 300 ml. of hot water as above. The insoluble ichthylepidin weighed 16 Gm. (N = 15.4 per cent, Molish test was negative.) The clear filtrate and washing were combined and concentrated to a thin syrup in vacuo. The syrup was dried in a current of warm air on a glass plate. The fish-scale gelatin (glue) weighed 15 Gm. (N = 16.3 per cent.)

Fish-scale gelatin and the ichthylepidin were quickly digested by both pepsin in 0.36 per cent of HCl or by trypsin at pH 7.9. The digested material did not give any precipitate with CCl_3COOH.

Amino Acid Analyses

Quantitative.—Nitrogen was determined by the Kjeldahl method; arginine, histidine, and lysine were isolated by the silver precipitation method, duplicate determinations[3] or determined microbiologically on two hydrolysates, if possible, at 10 different dilution levels[16]; tyrosine and tryptophane were determined by the Millon-Lugg method, four replicates[3]; phenylalanine was estimated by a modification of Kapeller-Adler's reaction, average of six determinations[3]; cystine and methionine were estimated by the Folin and Sullivan methods, respectively, average of four analyses[3]; threonine and serine were determined by the periodate oxidation procedure on four analyses.[3] Leucine, isoleucine, valine, glutamic acid, glycine and proline were estimated by microbiological methods.[17-20]

Qualitative.—Qualitative analyses for the amino acids in the various preparations were carried out by color tests[3] and by the filter paper chromatographic technic of Schönbein[21] and Goppelsroeder[22] as developed by Consden, Gordon, and Martin.[23] The technic of Consden, Gordon, and Martin was modified to the extent that the trough holding the developing solvents is placed at the bottom of the chamber. The solvents climb by capillary action up the paper for a distance of approximately 10 inches, past a supporting rod, and then down the

other side to the full length of the paper (23 inches).[24] The solvents employed most widely in this study were: water-saturated phenol, collidine, and a mixture of equal parts of collidine and 2,6-lutidine. Another very successful solvent was made from lutidine 55 parts, ethanol 20 parts, and water 25 parts, all by volume.

DISCUSSION AND RESULTS

The analytical results are shown in Table I. Human enamel protein may be classed as an eukeratin because of its insolubility, indigestibility with proteolytic enzymes, and its yield of basic amino acids after acid hydrolysis.[1] The low quantity of cystine, which is in agreement with previous investigations,[5, 8, 9, 10] does not vitiate this conclusion.[1, 3] In contrast to all other eukeratins which have been studied, enamel eukeratin is very highly calcified, in fact it is the most highly mineralized tissue in the body.

The protein component of "brown pellicle"[13] is also insoluble in water, dilute acids and alkalies, and in organic solvents. It is not digested by pepsin or by trypsin, but it cannot be classed as an eukeratin because of the yield of histidine, lysine, and arginine after hydrolysis (Table I). The proteins of brown pellicle may be a type of pseudokeratin or denatured mucoprotein which is deposited on the teeth from the oral fluids. Further differences in the amino acid composition of enamel protein and of brown pellicle are indicated by two-dimensional paper chromatograms, the results of which are summarized as follows:

Enamel (Human): threonine, alanine, serine, leucine-isoleucine, aspartic acid, and proline gave strong spots. Phenylalanine, valine, glycine and glutamic acid showed medium strength. Weak spots were given by arginine, lysine, tyrosine, cystine, and cysteic acid. Histidine, methionine, and hydroxyproline were absent or present in very small amounts. "Brown Pellicle": alanine, alone, gave a very strong spot. Medium spots were given by lysine, tyrosine, phenylalanine, methionine, leucine-isoleucine, and glutamic acid. Arginine, histidine, threonine, valine, aspartic acid glycine and cystine gave weak spots. Serine, proline, and hydroxyproline were not seen on the chromatograms.

Some of the amino acids yielded after hydrolysis of the proteins of herring scales, scale gelatin, and ichthylepidin are also given in Table I. In order to facilitate comparison of these collagens with pork skin gelatin, the amino acids in this protein are also given in the table.[3] It will be seen that there are a number of differences in the amino acid make-up of the gelatins obtained from herring scales and from pork skin. These differences in amino acid composition may explain, in part, the discrepancies found in the literature in the amino acid pattern of gelatins of undesignated origin.

Chromatograms showed marked qualitative similarity in the amino acid composition of the gelatins derived from herring scales and from human dentin. Thus, the histological and embryological conclusions, that the dentin of the "true" teeth is derived from the dentin of fish scales, are supported by amino

TABLE I

APPROXIMATE YIELD OF SOME AMINO ACIDS AFTER ACID HYDROLYSIS OF HUMAN ENAMEL KERATIN, "BROWN PELLICLE," HERRING SCALES, SCALE GELATIN, AND ICHTHYLEPIDIN
(Calculated to 16.0 Gm. of Nitrogen)

AMINO ACID IN GM.	HUMAN ENAMEL EUKERATIN	BROWN PELLICLE	HERRING SCALES	HERRING SCALE GELATIN	ICHTHYL-EPIDIN	PORK SKIN GELATIN
Nitrogen*	11.9%	11.8%	16.0 %	16.3 %	15.4 %	15.5 %
Arginine	9.7 Gm.	1.6 Gm.	6.7 Gm.	7.9 Gm.	5.4 Gm.	8.0 Gm.
Histidine	0.9 Gm.	0.6 Gm.	2.5 Gm.	1.1 Gm.	3.1 Gm.	1.0 Gm.
Lysine	2.8 Gm.	4.2 Gm.	3.7 Gm.	3.4 Gm.	3.7 Gm.	5.0 Gm.
Tyrosine			2.8 Gm.	0.4 Gm.	5.2 Gm.	0.7 Gm.
Tryptophane			0.3 Gm.	0.0 Gm.	0.6 Gm.	0.0 Gm.
Phenylalanine			2.9 Gm.			2.1 Gm.
Cystine	<1 Gm.	>1 Gm.	0.7 Gm.	0.2 Gm.	1.1 Gm.	0.1 Gm.
Methionine			2.8 Gm.	1.4 Gm.	3.0 Gm.	0.8 Gm.
Threonine			2.8 Gm.			0.8 Gm.
Serine			7.5 Gm.			3.4 Gm.
Leucine			3.6 Gm.			3.1 Gm.
Isoleucine			1.9 Gm.			1.7 Gm.
Valine			2.9 Gm.			2.8 Gm.
Glutamic Acid			7.2 Gm.			8.4 Gm.
Glycine			20.6 Gm.			23.6 Gm.
Proline			8.6 Gm.			15.3 Gm.

*Corrected for ash.

acid analysis. The amino acid patterns of herring scale gelatin and of dentin (human) as given in the chromatograms are summarized as follows: proline, alanine, glycine, arginine and serine gave strong spots. Phenylalanine, threonine, leucine-isoleucine, methionine, valine, lysine, glutamic acid and hydroxyproline gave medium spots. Weak spots were given by histidine and aspartic acid while cystine and tyrosine were absent or present in very small amounts.

Although embryological evidence[4] indicates that herring scales have developed from the placoid scales of the elasmobranchs by simple enlargement, no evidence for the presence of appreciable quantities of keratin was found even when 500 Gm. of scales were digested with pepsin and trypsin. The protein material (ichthylepidin) remaining after extraction of gelatin from herring scales consisted of thin glistening plates which, of course, were insoluble in hot water but quickly dissolved in warm dilute acid and were readily digested by pepsin and by trypsin. These properties and the presence of hydroxyproline in ichthylepidin, as well as the x-ray investigations of Astbury[25] on fish scales, indicate that ichthylepidin is a collagen-like protein.

The quantitative data given in Table I show differences in the amino acid pattern of ichthylepidin and of typical gelatins. The chromatograms showed similarities and differences in the amino acid patterns of ichthylepidin when compared to herring scale gelatin and human dentin. The results obtained on the two-dimensional chromatogram of herring ichthylepidin may be summarized as follows: alanine, glycine, proline, hydroxyproline, and serine strong. Phenylalanine, leucine-isoleucine, methionine, valine, arginine, histidine, lysine, tyrosine threonine glutamic acid, and aspartic acid gave medium spots, while cystine was weak.

SUMMARY

Physical and chemical studies on the protein (proteins) of human dental enamel showed it to be an eukeratin. Similar investigations on Manly's "brown pellicle" indicate that its protein moiety is not identical with the protein component of human enamel.

The proteins of herring scales were split into two major groups; the one had the physical and chemical properties of gelatins and was very similar to human dentin, the other (ichthylepidin) was not solubilized by boiling with water and may be classified as a collagen-like protein.

The relationships between embryological and chemical finding are discussed.

We are indebted to Dr. A. Severino for collecting the major portion of the "brown pellicle" used in these experiments, and to Dr. H. A. Sober for preparing some of the chromatograms, to Professor Walter C. Hess for the sample of purified human dentin, and to Dr. Charles Lane, Shark Industries, Salerno, Fla., for the shark denticles.

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DECALCIFICATION OF BONE AND TEETH UNDER VACUUM—A RAPID METHOD FOR PRODUCING HARD TISSUE PREPARATIONS

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THE time required for decalcification of histologic specimens must be considered a technical problem of great importance. For those doing experimental work, it is rather nervebreaking that several weeks often will elapse before slides are available. This delay is especially inconvenient because planning of new experiments depends to a great extent on observations gained from earlier ones.

The modification of the paraffin method for embedding has been a forward step, but even by using this method, it is practically impossible to have slides made in less than 45 days because decalcification takes from about 30 to 40 days if nitric acid is used. Trichloracetic acid requires about double that time. In order to shorten the time for decalcification, the author tried to carry out the same process under vacuum. The result was very promising. A rather large piece of a jaw with teeth which would have required 35 days was ready in less than 3 days. The equipment needed is shown in Fig. 1. If the water pressure is sufficiently high, a vacuum pump fixed to the water tap can be used. It must be constructed with a valve in order to avoid water flowing back into the bottles in case the pressure should temporarily decrease. As seen from Fig. 1, the bottles may be coupled in series making it possible to use the same pump for an indefinite number of bottles. These should be provided with rubber corks because ordinary corks are not sufficiently tight. For larger specimens containing bone, it is convenient to have bottles of larger size than those shown in the illustration to permit decalcification without changing acid.

Histological slides from bone and teeth decalcified under vacuum are in all cases of as high quality as those produced in the usual way and in many cases they seem to be better. This may be because the tissues are in contact with the acid for a shorter period of time. There is no indication of damage caused to tissues or cells because of the faster chemical process. The reason that dissolution of inorganic bone salts is accelerated under vacuum may be that the carbon dioxide evaporates more easily and new acid comes in contact with undissolved bone at an increased rate.

During the decalcification, bubbles containing carbon dioxide are seen rising continuously from the object. As soon as the decalcification is finished, this current of bubbles will stop. As a margin of safety it is recommended to continue the process from 12 to 24 hours after the bubbling has stopped.

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It is a common experience that the time required for decalcification may be shortened if nitric acid is used. This is because nitric acid usually is applied in a much stronger concentration than trichloracetic acid. Both have about the same degree of dissociation, and both are among the most dissociated acids known. As a rule they are used in 5 per cent solution, but the nitric acid usually is diluted by volume per cent, while the trichloracetic acid is diluted by weight per cent because of its crystalline form. If their molecular weight had been the same, the nitric acid (spec. weight 1.4) a priori would have been 1.4 times stronger. The molecular weight of trichloracetic acid is 163 and that of nitric acid is 63. This means that 163 grams of trichloracetic acid are required to give the same number of acid radicals as 63 grams of nitric acid.

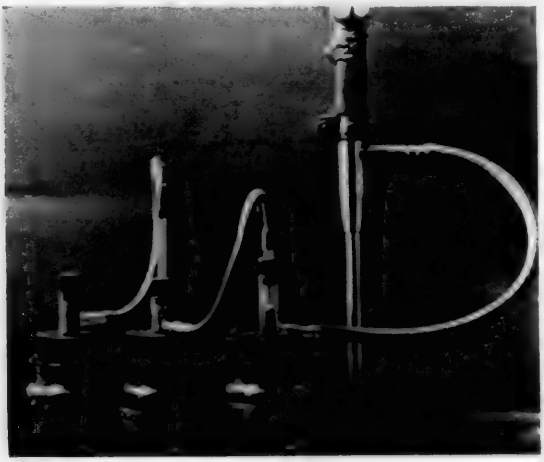

Fig. 1.

The same weight of both acids dissolved in equal quantities of water makes the nitric acid 2.6 (163 : 63 = 2.6) times more concentrated than the trichloracetic acid. Nitric acid is used in a concentration 3.64 (2.6 × 1.4) times stronger than trichloracetic acid. This may lend some explanation as to why better results are obtained with trichloracetic acid.

SUMMARY

By performing the decalcification under vacuum it has been possible to reduce the time required for this process to one-tenth. The rate of the process is proportional to the vacuum. For that reason it is recommended to use a rather high degree of vacuum (about 74 cm. Hg.).

The quality of the slides obtained in this way is usually better, no disadvantages have been observed after using the method for 6 months.

It is pointed out that nitric acid usually is applied in a concentration more than three times stronger than trichloracetic acid. This may explain the faster decalcification with nitric acid and the better result obtained with trichloracetic acid.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section

College of Dentistry, New York University—May 5, 1949*

Frances Krasnow, *Editor, New York Section*

1. Periarteritis nodosa: report of a case with involvement of the tongue. *Robert Gottsegen and Robert James Gorlin, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* A review of periarteritis nodosa from the standpoint of incidence, etiology, and pathology is presented and one case is cited in which typical vascular involvement has been found in the tongue. To our knowledge this is the first such case described in the literature.

2. Dental pulp changes associated with terminal pneumonia. *Alexander Seelig,† School of Dental and Oral Surgery, Columbia University, New York, N. Y.* Postmortem material of the teeth and jaws of two infants, 11 and 12 months of age, respectively, who had succumbed to pneumonia were made available for study. This material was prepared for histological investigation in the Vienna laboratories of Dr. E. Gottlieb. The pulps of incompletely formed teeth, which were still imbedded in the surrounding tissues, were studied histologically. The marrow of the jaws was included in this study to understand better the blood contents of the vessels of the pulp. In selecting incompletely developed and unerupted teeth, local or external environmental factors could reasonably be eliminated. The odontoblastic layer in the incisal region of the anterior teeth and in the area of pulp horns of the posterior teeth was found entirely destroyed and invaded by wandering cells. In the direction of the apical region of the teeth, the odontoblastic layer was present, but presented various stages of degeneration. Vacuolization of these cells was a fairly common observation, the nuclei were picnotic and small, the cytoplasm replete with strands, and the cell distended considerably beyond its normal size. In some instances the nucleus of the cell was absent. There was no evidence of leucocytes. The blood vessels were engorged with erythrocytes. These findings are associated with terminal pneumonia and give evidence that the pulp, as an end organ of the body, reacts to systemic changes.

3. Statistical methods of research in the determination of principles of dentistry. *Alfred J. Asgis,† College of Dentistry, New York University, New York, N. Y.* Dentistry has changed its biological and cultural makeup in the course of over a century of professional progress. It has assumed an important role in health conservation and social advancement. Dentistry may be defined as applied biology and sociology. The scope of dentistry in theory and

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†By invitation.

in practice has broadened. The study of its problems must be broadened accordingly. Research in the establishment of principles of dentistry is needed for a fuller understanding of the whole realm of dentistry, as well as for an understanding of any of its parts. Only with this understanding can we give sound direction to action. In the establishment of these principles a statistical method has been employed. It is acknowledged that a principle implies an original hypothesis, adequate verification, and the quality of generality and conformity to truth, at least in the pragmatic sense. Hypothesis may be verified by observation of practitioners, by special investigation, or by the experimental approach. In brief, a principle must be understood to mean a hypothesis so adequately tested by observation on experiment that it may intelligently be put forward as a means of understanding and a guide to action. The modified survey method was chosen in a study completed in 1939 to reveal fundamental principles. An opinionaire was used which contained statements of propositions or "principles." These related to facts of current conditions and practices and covered (a) persistent problems, (b) prevailing dental opinion, and (c) existing practices. Persistent problems were determined by a survey of dental literature. Prevailing opinion was determined by direct inquiry among 1,250 representative dentists. Current practices were determined by personal visits to dental schools and a review of data gathered from dental school catalogues. Professional, social, and educational principles were established by statistical analysis of 100 principles selected from 526 samples of statements. The analysis included initial tabulation of responses in terms of frequencies and determination of significant and weighted percentages.

4. OBSERVATIONS CONCERNING IN VIVO DISINTEGRATION OF SILICATE CEMENT RESTORATIONS. *Chester J. Henschel, Eastern Graduate Research Group, New York, N. Y.* Clinical observation of several thousand silicate cement restorations over a twenty-year period indicates that solubility in water or saliva is a negligible factor in their disintegration. While it is generally believed that silicate material "washes out," practically such action is limited principally to stagnant areas where food plaques accumulate and circulation of salivary fluids is at a minimum. In large interproximal silicates, the labial and lingual silicate surfaces exposed to both the most voluminous washing action of the saliva and to mechanical wear remains relatively intact. The deep "cupping" effect is noticeable mainly in the protected interproximal area. On cervical silicate fillings, the major disintegration occurs adjacent to the gingival sulcus where food plaques collect. It may be assumed, therefore, that silicate dissolution is due to acid action resulting from the degradation of ingested carbohydrates. Clinical records also disclose that the longevity of these restorations is somewhat proportionate to the relative immunity to dental caries of the particular individual. These observations indicate that the rate of silicate disintegration is influenced by the (1) rapidity and efficacy with which the natural salivary mechanism buffers adherent food debris, (2) amount and frequency of carbohydrate ingestion, and (3) relative cleanliness of the mouth whether it is natural or due to scrupulous and prompt effective hygienic methods.

5. THE ROLE OF THYROID-PARATHYROIDECTOMY TO CARIES INCIDENCE IN MALE SYRIAN HAMSTERS. *William Raebeck, Jr., New York, N. Y.* The role played by the endocrines in relation to the caries problem has been variously investigated with usually negative results. Yet Keyes in 1948, using the Syrian hamster, showed a decrease in the caries incidence in gonodectomized animals. The thyroid would appear to play a much greater role in regard to systemic influence upon the caries process. Since the hamster appears susceptible to a caries process similar to that of the human being, this animal was chosen. A group of male hamsters, half of which were thyroid-parathyroidectomized, were fed a caries-producing diet (Keyes, *N. Y. J. D.* 1948). The experiments ran from 95 to 196 days, with food and water consumption being controlled. A difference in total caries scores (Keyes, *J. D. Res.* 1944) appeared between the thyroid-parathyroidectomized and control groups of animals, showing a slight increase in caries incidence in the thyroid-parathyroidectomized animals. Another group of hamsters fed the caries-producing diet for 72 days showed a marked increase in total caries incidence as compared to the control hamsters in the thyroid-parathyroidectomized experiments. These animals were fed ad libitum. As a result, the possibility of individual strain variations to caries susceptibility in hamsters appears to exist. In addition, ad libitum feeding, as opposed to rational feeding, likewise may play a role in caries incidence.

6. THE EFFECT OF A PROTEIN FREE DIET ON THE TEETH AND PERIODONTIUM OF THE ALBINO RAT. *George Stein and Daniel E. Ziskin, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* Ten immature albino rats (45 to 60 grams) were fed a synthetic diet complete in all respects except that protein was omitted. At the end of a 3- to 4-week experimental period, the average weight loss was about 25 per cent. In a previous report epithelial changes in the tongue of these rats were reported. The cornified and dermal papillae of the tongue head disappeared, the epithelium became atrophic and covered by a thin hornified layer only.

Further study has revealed the following findings: The molars suffered changes in the odontoblastic layer consisting of focal interruptions of the regular layer. In areas involving ten to twenty cells the normal odontoblasts have disappeared and normal dentin apposition interrupted. The pulpal surface of the incisor dentin had an irregular outline. This change occurred more toward the incisal end involving the older odontoblasts. On the root surface of the molars resorption of various degrees was observed, very extensive in some instances. At some areas alveolar bone was resorbed opposite the site of root resorption and the normal arrangement of the principal fibres was disturbed. In two cases casein was added to the diet after marked clinical symptoms were manifest. Histological examination of the tongue showed reappearance of the papillae. Marked hyperemia of the molar pulps was seen with increased number of visible capillaries immediately under the odontoblastic layer. A group of animals were kept on a normal diet, except that the food intake was restricted to the extent that their weight remained on a similar level as the experimental animals. No tongue changes were seen. The teeth have not yet been examined.

7. CO-MEDICATION IN OPERATIVE DENTISTRY. *Carl R. Oman and Harold Sherman,* School of Dental and Oral Surgery, Columbia University, New York, N. Y.* Local anesthesia controls pain. Comedication controls the patient's apprehension. Co-medication is the use of a sedative drug in combination with a local anesthetic. Demerol Hydrochloride was combined with Novocain-Pontocaine-Cobefrin. Demerol Hydrochloride, a synthetic drug, is an analgesic, sedative, antispasmodic and antisialogogue. Controlled tests were run on ninety-three nonselected cases, 2.2 c.c. carpules of Novocain-pontocaine-Cobefrin were used. These contained 0, 25, 50 mg. of Demerol Hydrochloride.

The following conclusions were drawn: (1) Demerol Hydrochloride combined with Novocain-Pontocaine-Cobefrin provided effective local anesthesia in various operative procedures; (2) the above combination induced mild to marked sedation in almost all cases; (3) these drugs minimized the "anxiety syndrome" in several severe cases; (4) definite antisialogogue effect noted; (5) side actions are of minor significance—usually dizziness and sweating which occur during operating period; (6) fewer side actions from 25 mg. Demerol Hydrochloride than from Novocain-Pontocaine-Cobefrin alone; (7) average adult dose of Demerol Hydrochloride estimated at 35 mg. per 150 pounds; (8) sedation commences at 5 to 10 minutes following injection and is dissipated in 30 to 60 minutes; (9) very little risk of habituation when administered by dentist and in above doses; (10) escort desirable but not essential if proper precautions are observed; (11) comedication controls apprehension as well as pain; (12) co-medication should be employed in operative dentistry.

*By invitation.